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(54) Vascular endothelial cell growth factor C subunit

(57) Vascular endothelial cell growth factor C sub-unit DNA is prepared by polymerase chain reaction techniques. The DNA encodes a protein that may exist as either a heterodimer or homodimer. The protein is a mammalian vascular endothelial cell mitogen and as such is useful for the promotion of vascular development and repair. This unique growth factor is also useful in the promotion of tissue repair.

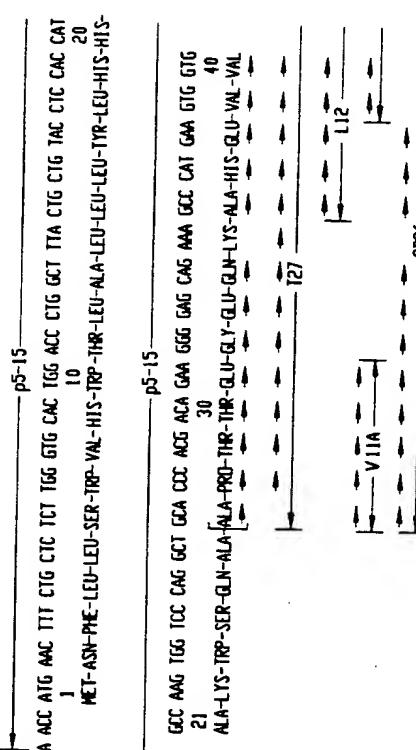


FIG.1A

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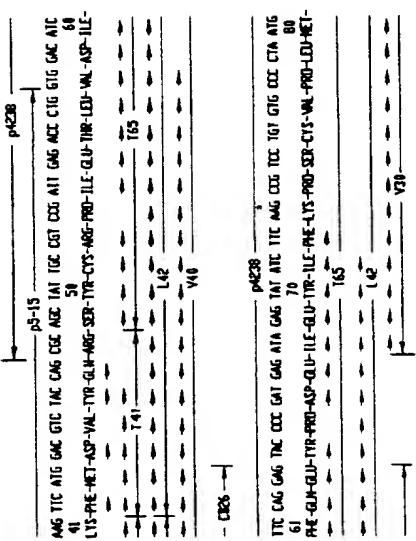


FIG. 1B

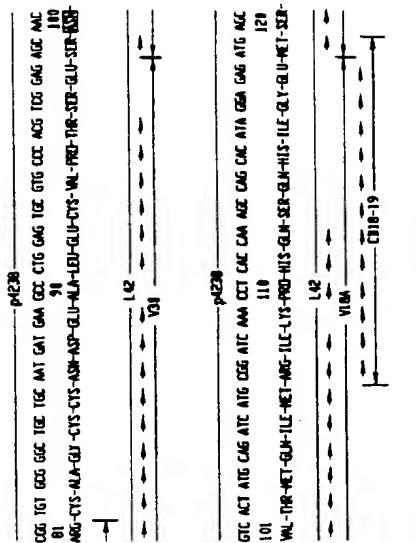
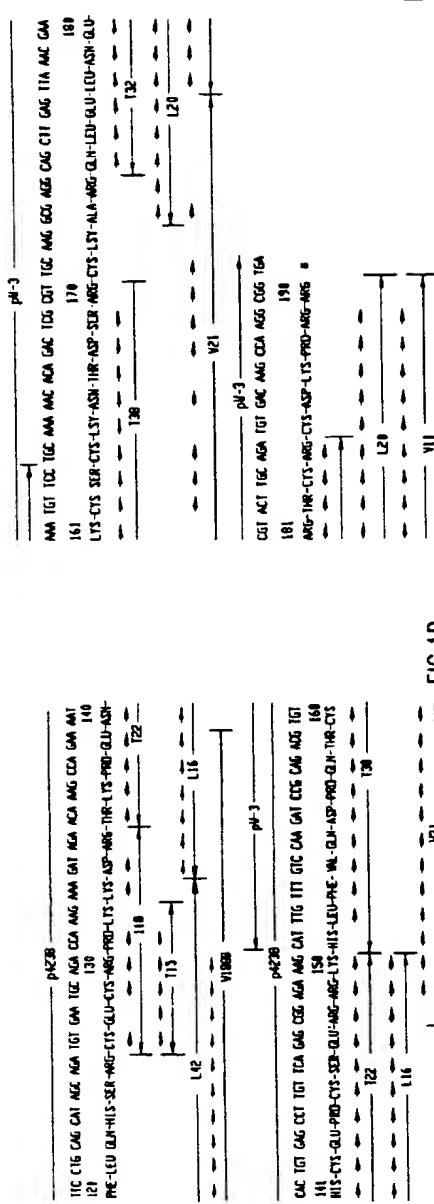


FIG. 1C



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BRIEF DESCRIPTION OF THE DRAWING

- Figure 1. Full length amino acid residue protein translation product and its cDNA coding sequence for VEGF AA subunit A plus polypeptide cleavage products used to determine the amino acid sequence.
- 5 Figure 2. Full length amino acid residue protein translation product and its cDNA coding sequence for VEGF AB subunit A plus polypeptide cleavage products used to determine the amino acid sequence.
- Figure 3. Full length amino acid residue protein translation product and its cDNA coding sequence for VEGF AB subunit B plus polypeptide cleavage products used to determine the amino acid sequence.
- 10 Figure 4. Full cDNA coding sequence and full length amino acid residue protein translation product for VEGF A 146 amino acid residue subunit SEQ ID NOS:23 & 33.
- Figure 5. Full cDNA coding sequence and full length amino acid residue protein translation product for VEGF A 190 amino acid residue subunit SEQ ID NOS:30 & 31.
- Figure 6. Full cDNA coding sequence and full length amino acid residue protein translation product for VEGF A 214 amino acid residue subunit SEQ ID NOS:34 & 35.
- 15 Figure 7. Full cDNA coding sequence and full length amino acid residue protein translation product for VEGF B 138 amino acid residue subunit SEQ ID NOS:36 & 37.
- Figure 8. Full cDNA coding sequence and full length amino acid residue protein translation product for VEGF B 158 amino acid residue subunit SEQ ID NOS:38 & 39.
- 20 Figure 9. Full cDNA coding sequence and full length amino acid residue protein translation product for VEGF C 154 amino acid residue subunit SEQ ID NOS:40 & 41.

BACKGROUND OF THE INVENTION

A new class of cell-derived dimeric mitogens with apparently restricted specificity for vascular endothelial 25 cells has recently been identified and generally designated vascular endothelial growth factors (VEGFs). The mitogen has been purified from: conditioned growth media of rat glioma cells, [Conn *et al.*, Proc. Natl. Acad. Sci. USA 87: 1323-1327 (1990)]; conditioned growth media of bovine pituitary folliculo stellate cells [Ferrara and Henzel, Biochem. Biophys. Res. Comm. 161: 851-858 (1989) and Gospodarowicz *et al.*, Proc. Natl. Acad. Sci. USA 86: 7311-7315 (1989)]. An endothelial cell growth factor isolated form mouse neuroblastoma cell line 30 NB41 with an unreduced molecular mass of 43-51 kDa and a reduced mass of 23-29 kDa has been described by Levy *et al.*, Growth Factors 2: 9-19 (1989). Connolly *et al.* (J. Biol. Chem. 264: 20017-20024 [1989]; J. Clin. Invest. 84: 1470-1478 [1989]) describe a human vascular permeability factor that stimulates vascular endothelial cells to divide *in vitro* and promotes the growth of new blood vessels when administered into healing rabbit bone grafts or rat corneas. An endothelial cell growth factor has been purified from the conditioned medium of 35 the AtT-20 pituitary cell line by Plouet *et al.*, EMBO Journal 8: 3801-3806 (1989). The growth factor was characterized as a heterodimer composed of subunits with molecular mass of 23 kDa. Leung *et al.* (Science 246: 1306-1309 [1989]), Keck *et al.* (Science 246: 1309-1312 [1989]) and Conn *et al.* (Proc. Natl. Acad. Sci USA 87: 2628-2632 [1990]) have described cDNAs which encode VEGF A which is homologous to the A and B chains of platelet-derived growth factor. Vascular endothelial growth factor I (VEGF I, VEGF AA) is a homodimer with 40 an apparent molecular mass of 46 kDa, with each subunit having an apparent molecular mass of 23 kDa. VEGF I has distinct structural similarities to platelet-derived growth factor (PDGF), a mitogen for connective tissue cells but not vascular endothelial cells from large vessels.

OBJECTS OF THE INVENTION

45 It is, accordingly, an object of the present invention to provide novel vascular endothelial growth factor C subunit DNA free of other mammalian DNA. Another object is to provide recombinant genes capable of expressing VEGF C subunit monomer or dimer. Another object is to provide vectors containing the DNA sequences for VEGF A or B plus C subunits. A further object is to provide a host cell transformed with a vector containing the DNA sequence for VEGF A or B plus C or VEGF C alone. It is also an object to provide a recombinant process for making VEGF C subunit. Another object is to provide a novel vascular endothelial cell growth factor which contains the C subunit. This may include heterodimers AC and BC and homodimer CC.

SUMMARY OF THE INVENTION

55 Vascular endothelial cell growth factor C subunit DNA is prepared by polymerase chain reaction techniques. The DNA encodes a protein that may exist as either a heterodimer or homodimer. The protein is a mammalian vascular endothelial cell mitogen and as such is useful for the promotion of vascular development and

repair. This unique growth factor is also useful in the promotion of tissue repair.

DETAILED DESCRIPTION

5 The present invention relates to a unique vascular endothelial cell growth factor (designated VEGF), isolated and purified from glioma cell conditioned medium, which exhibits mitogenic stimulation of vascular endothelial cells. Glioma is defined herein as any neoplasm derived from one of the various types of cells that form the interstitial tissue of the central nervous system including brain, spinal cord, posterior pituitary gland and retina. Consequently, the scope of the present invention is intended to include the unique growth factor and retina. Consequently, the scope of the present invention is intended to include the unique growth factor and

10 isolated and purified from any mammalian tissue or other cells including cell lines. Cell lines include, but are not limited to, glioma-derived cell lines such as C6, HS 683 and GS-9L; glioblastomas such as A-172 and T98G; neuroblastomas such as IMR-32 and SK-N-MC; neurogliomas such as H4; teratomas such as XB-2; astrocytomas such as U-87 MG and U-373 MG; embryonal carcinomas and non-transformed glial or astrocyte cell lines, and the human medulloblastoma line TE 671, with GS-9L and TE 671 being preferred. VEGF AB is present and can be isolated from rat tissue including ovary, heart and kidney. Anterior pituitary tumor cell lines such as GH3 and HS 199 may also be used. It is intended that VEGF of this invention can be obtained from any mammal species capable of producing VEGF, this includes, but is not limited to, rat and human.

Vascular endothelial cell growth factor may exist in various microheterogeneous forms which are isolated from one or more of the various cells or tissues described above. Microheterogeneous forms as used herein refer to a single gene product, that is a peptide produced from a single gene unit of DNA, which is structurally modified at the mRNA level or following translation. Peptide and protein are used interchangeably herein. The modified microheterogeneous forms will all have similar mitogenic activities. Biological activity and biologically active are used interchangeably and are herein defined as the ability of VEGF to stimulate DNA synthesis in target cells including vascular endothelial cells as described below which results in cell proliferation. The modifications may take place either *in vivo*, or during the isolation and purification process. *In vivo* modification results from, but is not limited to, proteolysis, glycosylation, phosphorylation, deamidation or acetylation at the N-terminus. Proteolysis may include exoproteolysis wherein one or more terminal amino acids are sequentially, enzymatically cleaved to produce microheterogeneous forms which have fewer amino acids than the original gene product. Proteolysis may also include endoproteolytic modification that results from the action of endoproteases which cleave the peptide at specific locations within the amino acid sequence. Similar modifications can occur during the purification process which also results in production of microheterogeneous forms. The most common modification occurring during purification is proteolysis which is generally held to a minimum by the use of protease inhibitors. Under most conditions one or more microheterogeneous forms are present following purification of native VEGFs. Native VEGFs refers to VEGF isolated and purified from cells that produce VEGFs.

35 Vascular endothelial cell growth factor may also exist in various alternatively spliced forms which is defined herein as the production of related mRNAs by differential processing of exons and introns. Exons are defined as those parts of the DNA sequence of a eukaryotic gene that code for the final protein product. It is also intended that the present invention includes VEGF subunits A, B and C which are defined as comprising the full length translation products of all alternatively spliced mRNAs made from the gene encoding the subunits and their corresponding mature amino acid sequences generated by proteolytic removal of the amino terminal secretory leader amino acid sequences. It is further intended that the invention only include those microheterogeneous and alternatively spliced VEGF subunits which when in the dimeric form exhibit biological activity such as vascular endothelial cell stimulation as discussed below.

40 Glioma cells such as the rat cell line GS-9L are grown to confluence in tissue culture flasks, about 175 cm², in a cell culture medium such as Dulbecco's Modified Eagle's Medium (DMEM) supplemented with about 10% newborn calf serum (NCS). When the cells reach confluence the culture medium is removed, the cell layers are washed with Ca⁺⁺, Mg⁺⁺-free phosphate buffered saline (PBS) and are removed from the flasks by treatment with a solution of trypsin, about 0.1%, and EDTA, about 0.04%. The cells, about 1 x 10⁸, are pelleted by centrifugation, resuspended in about 1500 ml of DMEM containing about 5% NCS and plated into a ten level cell factory (NUNC), 6,000 cm² surface area. The cells are incubated for about 48 to about 96 hours, with 72 hours preferred, at about 37° C in an atmosphere of about 5% CO₂. Following incubation the medium is removed and the cell factories are washed about 3 times with PBS. About 1500 ml of fresh culture media is added containing about a 1:2 mixture of Ham's-F12/DMEM containing about 15 mM Hepes, pH about 7.4, about 5 µg/ml insulin, about 10 µg/ml transferrin and with or without about 1.0 mg/ml bovine serum albumin. This medium is replaced with fresh medium after about 24 hr and collected every 48 hr thereafter. The collected conditioned medium is filtered through Whatman #1 paper to remove cell debris and stored at about -20° C.

45 The GS-9L conditioned medium is thawed and brought to pH 6.0 with 1 M HCl. The initial purification step consists of cation exchange chromatography using a variety of cation exchangers on a variety of matrices such

as CM Sephadex C-50, Pharmacia Mono S, Zetachrom SP and Polyaspartic Acid WCX (Nest Group) with CM Sephadex C-50 (Pharmacia) being preferred. The VEGF-containing culture medium is mixed with CM Sephadex C-50 at about 2 gm per about 20 L of the conditioned medium and stirred at low speed for about 24 hr at 4° C. The resin is allowed to settle and the excess liquid is removed. The resin slurry is packed into a column and the remaining culture medium is removed. Unbound protein is washed from the column with 0.05 M sodium phosphate, about pH 6.0, containing 0.15 M NaCl. The VEGF AB is eluted with about 0.05 M sodium phosphate, about pH 6.0, containing about 0.6 M NaCl.

The active fractions collected from the CM Sephadex C-50 column are further fractionated by lectin affinity chromatography for additional purification of VEGF AB. The lectins which may bind VEGF AB include, but are not limited to, lectins which specifically bind mannose residues such as concanavalin A and lens culinaris agglutinin, lectins which bind N-acetylglucosamine such as wheat germ agglutinin, lectins that bind galactose or galactosamine and lectins which bind sialic acids, with concanavalin A (Con A) being preferred. A 0.9 cm diameter column containing about 5 ml packed volume of Con A agarose (Vector Laboratories) is washed and equilibrated with about 0.05 M sodium acetate, about pH 6.0, containing about 1 mM CaCl₂, about 1 mM MnCl₂ and about 0.6 M NaCl. The unbound protein is washed from the column with equilibration buffer. The VEGF AB is eluted with about 0.1 M NaCl buffer containing about 0.32 M α-methyl mannoside and about 0.28 M α-methyl glucoside.

The VEGF AB active eluate from the Con-A column is applied to a Polyaspartic Acid WCX cation exchange high performance liquid chromatography (HPLC) column, 4.6 mm x 250 mm, pre-equilibrated in about 0.05 M sodium phosphate buffer, pH 6.0. The column is eluted with a linear gradient of about 0 to 0.75 M NaCl in the phosphate buffer over about 60 minutes. The flow rate is maintained at about 0.75 ml/min collecting 0.75 ml fractions. Vascular endothelial cell growth factor AB activity is present in fractions eluting between approximately 21.7 and 28.5 ml.

The active fractions eluted from the polyaspartic WCX column that contain VEGF AB are pooled, adjusted to about pH 7.0 and loaded onto a 1 x 10 cm column of Pharmacia Chelating Sepharose 6B charged with an excess of copper chloride and equilibrated in about 0.05 M sodium phosphate, about pH 7.0, containing about 2 M NaCl and about 0.5 mM imidazole (A buffer). VEGF AB is eluted from the column with a gradient from 0-20% B over 10 minutes, 20-35% B over 45 minutes and 35-100% B over 5 minutes at a flow rate of 0.3 ml/min, where B buffer is 0.05 M sodium phosphate, pH 7.0, containing about 2 M NaCl and 100 mM imidazole. The active fractions containing VEGF AB activity eluted between about 12.6 and 22.8 ml of the gradient effluent volume.

The pooled fractions containing VEGF AB activity eluted from the metal chelate column are loaded onto a 4.6 mm x 5 cm Vydac C₄ reverse phase HPLC column (5 μm particle size) previously equilibrated in solvent A [0.1% trifluoroacetic acid (TFA)]. The column is eluted with a linear gradient of about 0 to 30% solvent B over 15 minutes, 30% B for an additional 15 minutes, then 30-45% B over 22.5 minutes and finally 45-100% B over 5.5 minutes. Solvent B consists of solvent A containing 67% acetonitrile (v/v). The flow rate is maintained at about 0.75 ml/min and fractions are collected every minute. The homogeneous VEGF AB elutes from the C₄ column under these conditions at between about 32 and about 38 ml of the gradient effluent volume.

Purity of the protein is determined by sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis (PAGE) in 12.5% crosslinked gels using the technique of Laemmli, Nature 227:680-684 (1970). The silver stained gels show VEGF AB to consist of one band under non-reducing conditions with an approximate apparent molecular mass of about 58,000 daltons. When a sample containing the microheterogeneous forms of VEGF AB is separated under reducing conditions it migrates as two about 23 kilodalton (kDa) subunits. The purification process results in VEGF AB that is essentially free of other mammalian cell products, such as proteins. Recombinantly derived VEGF AB will also be free of mammalian cell products.

Biological activity is determined by mitogenic assay using mammalian vascular endothelial cells. Human umbilical vein endothelial (HUVE) cells are plated on gelatin-coated dishes at a density of about 5000 cells per well in about 500 μl of Medium 199 (M199) containing about 20% heat-inactivated fetal calf serum (FCS). Samples to be assayed are added at the time of plating. The tissue culture plates are incubated at about 37° C for about 12 hours and about 2 microcuries of tritiated thymidine (NEN, 20 Ci/mmol) is added per ml of assay medium (1.0 μCi/well). The plates are incubated for a further 60 hr, the assay medium is removed and the plates are washed with Hanks balanced salt solution containing about 20 mM Hepes, about pH 7.5, and about 0.5 mg/ml bovine serum albumin. The cells are lysed and the labelled DNA solubilized with about 200 μl of a solution containing about 2 gm of sodium carbonate and about 400 mg sodium hydroxide in about 100 ml water. The incorporated radioactivity was determined by liquid scintillation counting. The concentration of VEGF which elicited a half-maximal mitogenic response in HUVE cells was approximately 2 ± 1 ng/ml. The glycosaminoglycan heparin, which is required in these assays at a level of 10-100 μg/ml to promote a response to a positive control, acidic fibroblast growth factor, does not enhance mitogenic stimulation of these cells by VEGF AB.

A purified about 1-2 µg sample of VEGF AB is reduced in about 0.1 M Tris, about pH 9.5, with about 0.1% EDTA, about 6 M guanidinium chloride and about 20 mM dithiothreitol for about 2 hr at about 50°C. The reduced protein is carboxymethylated for about 1 hour in a solution containing about 9.2 µM of unlabelled and 2.8 µM of ¹⁴C-iodoacetic acid in about 0.7 M Tris, about pH 7.8, and about 0.1% EDTA and about 6 M guanidinium chloride. The protein is carboxymethylated for about 1 hr at room temperature. The protein is isolated after reduction and carboxymethylation by reverse phase HPLC chromatography on a Vydac C₄ column, about 4.6 mm x 5 cm. The protein subunits are loaded onto a column pre-equilibrated with about 0.1% TFA and eluted by a 45 ml linear gradient from about 0.1% TFA to 0.1% TFA/67% acetonitrile at a flow rate of about 0.75 ml/min. The reduced and carboxymethylated protein eluted as two peaks at approximately 23 and 25 ml with the proportion being approximately equal as determined by monitoring absorbance at 210 nm.

Samples of the reduced and carboxymethylated monomers are applied to polybrene-coated glass fiber filters and their N-terminal sequences are determined by Edman degradation in an ABI gas phase microsequencer in conjunction with an ABI 120A on line phenylthiohydantoin analyzer following the manufacturers instructions. The protein showing the peak of absorbance eluting at approximately 25 ml (A subunit or monomer) yielded an amino terminal sequence of: SEQ ID NO:1

Ala Pro Thr Thr Glu Gly Glu Gln Lys Ala His Glu Val Val

which is identical to the A chain monomers of VEGF AA, Conn et al., Proc. Natl. Acad. Sci. USA 87: 2628-2632 (1990). The peak of absorbance eluting at approximately 23 ml (B subunit or monomer) yielded an N-terminal sequence of: SEQ ID NO:2

Ala Leu Ser Ala Gly Asn Xaa Ser Thr Ser Thr Glu Met Glu Val Val
Pro Phe Asn Glu Val

plus a nearly equal amount of a truncated form of the same sequence missing the first three amino acid residues. The missing Xxx residue corresponds to an Asn residue in the cloned cDNA, see below. Since this missing Asn occurs in a classical Asn Xxx Ser/Thr N-glycosylation sequence it is presumed to be glycosylated. The A subunit and the total of both B subunits are recovered in nearly equal amounts supporting the interpretation that the two peptides combine to form an AB heterodimer in VEGF AB.

A sample of the A monomer was treated with either the protease trypsin which cleaves polypeptides on the C-terminal side of lysine and arginine residues or Lys C which cleaves polypeptides on the C-terminal side of lysine by procedures well known in the art. The peptides are isolated by reversed phase - HPLC(RP-HPLC).

The amino acid sequences of the isolated peptides are determined using the Edman degradation in the ABI gas phase sequencer in conjunction with the ABI 120 A on line phenylthiohydantoin analyzer following manufacturer's instructions. The amino acid sequences are shown in Figure 1.

Reduced and carboxymethylated A monomer is dried and solubilized in about 0.7 M Tris, about pH 7.8, about 6 M guanidinium chloride containing about 0.1% EDTA. V8 protease is added in 0.1 M ammonium bicarbonate buffer, about pH 8.0, and the mixture is incubated for about 48 hr at about 37°C. The protease cleaves predominantly on the carboxyl terminal side of glutamic acid residues. The resulting polypeptides were resolved by C₁₈ RP-HPLC as above.

The reduced and carboxymethylated A subunit protein solution is adjusted to a pH of about 6.8 with 6 N HCl and dithiotreitol is added to a final concentration of 2 M for reduction of any methionine sulfoxide to methionine residues. After about 20 hr of reduction at about 39°C the protein is repurified by C₄ HPLC. The product is dried and cleaved on the carboxyl terminal side of methionine residues by 200 µl of 40 mM cyanogen bromide in about 70 % (v/v) formic acid under an argon atmosphere at about 20°C for about 24 hr in the dark. The cleavage products are resolved by C₁₈ RP-HPLC. The amino acid sequence is shown in Figure 1, see Conn et al., Proc. Natl. Acad. Sci USA 87:2628-2632 (1990).

The full length 190 amino acid residue protein translation product of the VEGF AB, A monomer or subunit, which is now known to be identical with the VEGF AA, A monomer, and its cDNA coding sequence are shown in Figures 2 and 6. The mature amino terminus begins at residue 27, immediately following a typical hydrophobic secretory leader sequence. A single potential N-glycosylation site exists at Asn₁₀₀. Most (143 amino acid residues) of the 164 residues of the reduced and carboxymethylated mature subunit including the amino terminus and HPLC reversed phase-purified products of tryptic (T), Lys-C (L), Staphylococcus aureus V8 protease (V8) and cyanogen bromide (CB) cleavages, were determined by direct microsequencing (Applied Biosystems 470A) using a total of 5 µg of protein. All residues identified by amino acid sequencing are denoted by arrows pointing to the right either directly beneath the mature processed sequence following the bracket at residue 27 for the amino terminal determination of the whole subunit or, for residues identified from the polypeptide cleavage products, above the double-headed arrows spanning the length of the particular polypeptide. One listed pair of polypeptides, V18A and V18B, was sequenced as a mixture and, therefore, are only confirmatory of the cDNA-deduced amino acid sequence, see Figures 1 and 5.

Samples of the reduced and carboxymethylated pure VEGF AB, A and B monomers, were each digested

The novel growth factors of this invention are useful for the coverage of artificial blood vessels with vascular endothelial cells. Vascular endothelial cells from the patient would be obtained by removal of a small segment of peripheral blood vessel or capillary-containing tissue and the desired cells would be grown in culture in the presence of VEGF and any other supplemental components that might be required for growth. After growth of adequate numbers of endothelial cells in culture to cover a synthetic polymeric blood vessel the cells would be plated on the inside surface of the vessel, such as fixed umbilical vein, which is then implanted in the patient.

The novel method for the stimulation of vascular endothelial cells companies treating a sample of the desired in need of such treatment. The novel method for the stimulation of vascular endothelial cells companies treating a sample of the desired in need of such treatment.

The ability of the various species of VEGF to stimulate the division of vascular endothelial cells makes this protein in all microheterogeneous forms and alternative splicing forms useful as a pharmaceutical agent. The protein used here in is intended to include all microheterogeneous forms as previously described. The protein can be used to treat wounds of mammals including humans by the administration of the novel protein to patients.

The expressed proteins (homodimers or heterodimers) are isolated and purified by standard protein purification techniques. Plasmids containing a single subunit species may be used to construct a subunit vector.

The high C₅ content and glycosylation sites of the A, B and C subunits along with the structure of the homodimeric proteins suggest that expression of biologically active proteins can be carried out in animal cells. Expression may be carried out in Chinese hamster ovary (CHO) cells with the cloned VEGF DNA construct selected and heterodimers may be carried out in animal cells. Expression of biologically active proteins such as the heterodimers containing diphyside reductase (dfrh-) into dfrh- CHO cells with the cloned VEGF DNA construct selected with the gene encoding diphyside reductase (dfrh-) into dfrh- CHO cells. see Sambrook et al. Transfomants expressing dfrh are selected on media lacking nucleosides and are exposed to a stable cell line capable of expressing methotrexate. The dfrh and VEGF genes are thus complemented leading to a stable cell line expressing high levels of VEGF. The plasmid is designed to encode either an A subunit, a B subunit or a C subunit or a combination of any two of these subunits. The two DNAs are operably attached so that the protein produced will be dimeric and will have VEGF biological activity. Operably attached refers to an appropriate sequence arrangement of nucleotide segments of genes such that the desired protein will be produced by cells containing an expression vector containing the operably attached genes. CDNA segments or nucleotides containing an expression vector containing the operably attached genes, CDNA segments or nucleotides of nucleotide sequences, CDNA segments of genes such that the desired protein will be produced by cells containing an expression vector containing the operably attached genes.

by Linnemann et al., Europerat. Fribm Applpharmacol., 1983, 223-3 (Phamacia) as modified as by Linnemann et al., and exp-

GS-9-L conditioned medium, from Example 1, was thawed and brought to pH 6.0 with 1 M HCl. Two grams

Carboxymethyl-Sephadex Chromatography of VEGF AA and VEGF AB

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EXAMPLE 2

5-9L cells were grown to confluence in 175 cm² tissue culture flasks in Dulbecco's Modified Eagle's Medium/10% newborn calf serum (DMEM/NCS). At confluence the medium was decanted from the flasks, the flasks were washed with calcium and magnesium free phosphate buffered saline (PBS) and the cells were removed by treatment with a 1X solution of trypsin/EDTA (Gibco). The cells (1×10^6) were pelleted by centrifugation, resuspended in 1500 ml of DMEM/5% NCS and plated into a ten level (6000 cm² surface area) cell factory (NUNC). After 72 hours incubation at 37°C in a 5% CO₂ atmosphere the medium was decanted and the cell factories were washed 3 times with PBS. The cells were refed with 1500 ml of a 1:2 mixture of Ham's F-12/DMEM containing 25 mM HEPES, pH 7.4, 5 µg/ml insulin, 10 µg/ml transferrin and 1.0 mg/ml bovine serum albumin. This medium was changed with fresh F-12/DMEM after 24 hours and collected every 48 hours after that. The conditioned medium was filtered through a Whatman #1 paper to remove cell debris and stored frozen at -20°C.

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Preparation of Medium Conditioned By GS-9L Cells

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EXAMPLE 1

The following examples illustrate the present invention without, however, limiting the same thereto.

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For non-topical applications the VEGF is administered in combination with pharmacaceutically acceptable carriers or diluents such as, phosphate buffer, saline, phosphatase buffered saline, ringers solution, and the like, in a pharmaceutical composition, according to standard pharmaceutical practice. For topical application, various pharmaceutical formulations are useful for the administration of the active compound of this invention. Such formulations include, but are not limited to, the following: ointments such as hydrophilic petrolatum or polyethylene glycol ointment pastes which may contain gels; solutions such as alcoholic solutions or aqueous solutions; gels such as aluminum hydroxide or sodium alginate gels; solutions such as animal albumins; collagen such as human or animal collagen; celluloses such as alkyl celluloses, hydroxy alkyl celluloses and alkylhydroxyalkyl celluloses; celluloses such as tetronic 150B; and alginates such as sodium alginate.

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Alternatively, tubular supports are coated *in vitro* with VEGF prior to implantation into a patient. Following implantation endothelial cells migrate into and grow on the artificial surface. Prior coating of the artificial vessel either covalescently or noncovalescently, with proteins such as fibrin, collagen, fibronectin or laminin would be performed to enhance attachment of the cells to the artificial surface. The cell-lined artificial vessel would then be surgically implanted into the patient and, being lined with the patient's own cells, would be immunologically compatible. The non-thrombogenic endothelial cell lining should decrease the incidence of clot formation on the surface of the artificial vessel and thereby decrease the tendency of vessel blockage or embolism elsewhere. The novel proteins are also used for the production of artificial vessels. Vascular endothelial cells would be grown in the presence of VEGF as outlined above. The smooth muscle would be grown in culture by procedures well known in the art. A tubular mesh matrix of a biocompatible polymer (either a synthetic polymer, with or without a coating of proteins, or a non-immunogenic biopolymer) would be grown in culture by threads on the interior surface. Once the endothelial cells form a confluent monolayer on the inside surface and multiple layers of smooth muscle cells cover the outside, the vessel is implanted into the patient.

The novel peptides can also be used for the induction of tissue repair. The pure VEGF would be used to induce and healing of surface wounds the formulation would be applied directly at a rate of about 1 ng to about 1 mg/cm²/day. For vascular repair VEGF is given intravenously at a rate of about 1 ng to about 100 ng/kg/day or body weight. For muscular repair, the formulation would be released directly into the region to be neovascularized either from implanted vascular growth, the formulation would be released from pumps or implants. The release rate in either case is about 10 ng to about 100 ng/day/cm³.

Human umbilical vein endothelial cells (HUVE) were plated on gelatin-coated 48 well tissue culture dishes

Mitogenetic Assays

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EXAMPLE 7

The fractions containing VEGF AB activity pooled from the metal chelate column, Example 5 were loaded onto a 4.6 mm x 5 cm Vydac C₁₈ reverse phase HPLC column (5 μ m particle size) equilibrated in solvent A (0.1% trifluoroacetic acid (TFA)). The column was eluted with a gradient of 0-30% solvent B over 15 minutes, 30% B for an additional 15 minutes, then 30-45% B over 22.5 minutes and finally 45-100% B over 5.5 minutes where solvent B = A containing 67% acetonitrile. The flow rate was maintained at 0.75 ml/min. The active VEGF AB fractions eluting between approximately 32.2 and 37.5 ml of the gradient effluent volume were pooled.

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Reverse Phase Chromatography

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EXAMPLE 6

The active fractions eluted from the poly(aspartic acid) WDX column, Example 4, that contain VEGF-AB were pooled, adjusted to pH 7.0 and loaded onto a 1 x 10 cm column of Pharmacia Chelating Sepharose 6B charged with an excess of copper chloride and equilibrated in 0.05 M sodium phosphate, pH 7.0, containing 2 M NaCl and 0.5 mM imidazole (a buffer). VEGF-AB was eluted from the column with a gradient from 0-20% B over 10 minutes, 20-35% B over 45 minutes and 35-100% B over 5 minutes at a flow rate of 0.3 ml/min, where buffer was 0.05 M sodium phosphate, pH 7.0, containing 2 M NaCl and 100 mM imidazole. The active fractions containing VEGF-AB activity eluting between 12.6 and 22.8 ml of the gradient volume were pooled.

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Metal Chelate Chromatography

EXAMPLE 5

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The active eluate from the Cen A column, Example 3, was applied to a 25 cm x 4.6 mm poly(aspartic acid) WCX column exchange HPLC column (Nest Group) pre-equilibrated in 0.05 M sodium phosphate buffer, pH 6.0. The column was eluted with a linear gradient of 0 to 0.75 M NaCl in this buffer over 60 minutes at a flow rate of 0.75 ml/min collecting 0.75 ml fractions. VEGF AB activity present in fractions eluting between approximately 21.7 and 28.5 ml were pooled.

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Polyaspartic Acid WCX HPLC Cation Exchange Chromatography of VEGF AA and VEGF AB

EXAMPLE 4

02

A 0.9 cm diameter column containing about 5 ml of packed Con A agarose (VectaLabs) was equilibrated with 0.05 M sodium acetate, pH 6.0, containing 1 mM Ca^{++} , 1 mM Mn^{++} and 0.6 M NaCl. The active eluate from the CM Sephadex G-50 column, Example 2, was applied to the Con A agarose and unbound protein was washed from the column with equilibrium buffer. The column was then rinsed with three column volumes of 0.05 M sodium acetate, pH 6.0, containing 1 mM Ca^{++} , 1 mM Mn^{++} and 0.1 M NaCl. Bound protein was subsequently eluted from the column by application of this buffer supplemented with 0.32 M α -methyl mannoside and 0.28 M α -methyl glucoside.

EXAMPLE 3

of CM Sephadex C-50 cation exchange (Pharmacia) resin preequilibrated in PBS adjusted to pH 6.0 with 1 N HCl were added to 20 litres of conditioned medium. The mixture was stirred at low speed for 24 hours at 4°C. The resin was then allowed to settle and the medium was siphoned off. The remaining resin slurry was packed into a 3.0 cm diameter column and any remaining medium was allowed to drain off. Unbound protein was washed off the column with 0.05 M sodium phosphate, pH 6.0, containing 0.15 M NaCl. Vacular endothelial growth factor activity was eluted from the column with a subsequent wash of 0.05 M sodium phosphate, pH 6.0, containing 0.6 M NaCl.

This analysis also sequences the isolated peptides in the AB1 120 A on-line phenylthiohydantoin analyzer (Applied Biosystems Int.). The peptide sequences are shown in the following figures 2 and 3. The amino acid sequence

in acid-washed 10×75 mm glass tubes. Lys C Protease (50 ng, Boehringer Mannheim), an enzyme that cleaves on the carboxyl terminal side of lysine residues, was added to each tube in 100 μ l of 25 mM Tris, pH 8.5, 0.1% EDTA. The substrate protein subunits were separately digested at 37°C for 8 hours and the resulting polypeptides resolved by reversed phase HPLC chromatography on a 4.6 mm x 25 cm Vydac C₁₈ column equilibrated in 0.1% TFA at a flow rate of 0.75 ml/min at 20°C. Individual peaks were manually collected and stored in this elution solution at 4°C.

Samples of the two protein fiber subunits isolated after reduction and carboxymethylation were each applied to polyacrylene-coated glass fiber filters and their N-terminal sequences were determined by Edman degradation in an ABI gas phase microsequencer in conjunction with an ABI 120A on line phenylthiohydantoin analyzer following manual sequencing instructions. The peak of absorbance eluting at approximately 25 ml (A subunit) yielded an amino terminal sequence Ala Pro Thr Thr Gly Glu Glu Lys Ala His Val Val SEQ ID NO: 1 identified to VEGF AA. The peak of absorbance eluting at approximately 23 ml (B subunit) yielded the N-terminal sequence Ala Ser Ala Gly Asn Xaa Ser Thr Gly Met Val Val Pro Pro Asn Glu Val SEQ ID NO: 2 plus a nearyl equal amount of a truncated form of the same sequence missing the first three residues. The missing X residue corresponds to an Asn in the cloned sequence. Since this missing Asn occurs in a classical Asn-X-Ser/Thr-N-glycosylation sequence it is presumed to be glycosylated. The A and sum of the B chain peptides were recovered in nearly equal amounts supporting the interpretation that the two peptides combine to form an AB heterodimer in VEGF II.

homogeneous protein in reversed phase C₁₈ in the aqueous trifluoroacetic acid (TFA)/acetonitrile mixture used to elute the previous study described. Aliquots of the purified protein (1-2 µg) were vacuum evaporated to dryness in acid-wash 10 x 75 mm glass tubes and reduced for 2 hours at 50°C in 100 µl of 1 M Tris buffer, pH 9.5, and 6 M guanidinium chloride containing 0.1% EDTA and 20 mM dithiothreitol (CibaIcIchem, Ultro grade) under an argon atmosphere. The reduced protein was subsequently carbonylmethylated for 1 hour at 20°C by the addition of 100 µl of 0.7 M Tris, pH 7.8, containing 0.1% C₁₈-acetic acid (17.9 µCi/mmol, Amersham). After completion of the carbonylmethylation, the mixture was loaded directly onto a 4.6 mm x 5.0 cm Vydac C₁₈ column which had been preequilibrated in 0.1% TFA. The reduced and carboxymethylated protein was reperfused by elution with a 45 minute linear gradient of 0 to 67% (v/v) acetonitrile in 0.1% TFA at a flow rate of 0.75 ml/min and stored in this elution solution at 4°C. The reduced and carboxymethylated protein eluted as two peaks at approximately 23 and 25 ml that were of approximately equal area as determined by monitoring absorbance at 210 nm.

Furnty of the protein under non-reducing conditions was determined by SDS-PAGE in 12.5% crosslinked gels according to the method of Laemmli, Nature 227: 680-685 (1970). The silver-stained gel contained a single band with an apparent mass of approximately 58 KDa. VEGF AB migrated in SDS-PAGE under reducing conditions in 15% crosslinked gels as a broad silver-stained band with molecular mass of approximately 3 KDa.

Utility And Protein Structural Characterization of VEGF AB

EXAMPLE 8

at a density of 5000 cells/well in 500 μ l of Medium 199 containing 20% fetal calf serum (FCS). Samples to be assayed were added at the time of plating. The tissue culture plates are incubated at 37°C for 12 hours and 2 microcures of titrated thymidine (NEN, 20 Ci/mmol) was added per ml of assay medium (1.0 μ Ci/well). The plates were incubated for a further 60 hr, the assay medium was removed and the plates were washed with Hanks balanced salt solution containing 20 M Hepes, pH 7.5, and 0.5 mg/ml bovine serum albumin. The cells were lysed and the labelled DNA solubilized with 200 μ l of a solution containing 2 gm of sodium carbonate and 400 mg sodium hydroxide in 100 ml water. The incorporated radioactivity was determined by liquid scintillation counting.

5. CAGAGAATTCGACCA[AG]TC[N]GT[AG]TT[TCC]TT
 142.2 5. TTGTCGACCT[TC]ATGGA[TC]GT[N]TA[TC]CA 3.
 SEQ ID NO:3
 T383B
 [AG]CA 3. SEQ ID NO:4
 where N=ACGT
 25
 Poly A+ RNA was isolated from GS-9L cells using the Fast Track RNA isolation kit from Invitrogen and the protocol provided. First strand cDNA synthesis was performed as follows:
 1 μg of GS-9L RNA was annealed to 1 μg of adapter primer T117,
 2.5 μl 100 mM DTT
 2.5 μl 10 mM each DATP, DCTP, DCTP, dTTP
 0.6 μl 15 units RNasin
 2.5 μl 40 mM Pyrophosphate
 1.5 μl 15 units reverse transcriptase
 and the reaction was incubated at 42°C for 1 hour, then diluted to 1 ml in 10 mM Tris-HCl / mM EDTA,
 pH 7.5.
 30
 5. GACTCGAGCATCCTGACCA[AG]TC[N]GT[AG]TT[TCC]TT
 1 μg of GS-9L RNA was annealed to 1 μg of adapter primer T117,
 2.5 μl 10X buffer (500 mM Tris-HCl pH 8.3, 750 mM KCl, 100 mM MgCl₂, 5 mM spermidine)
 3.0 μl Water
 70°C for 5 min, followed by cooling to room temperature. To this reaction was added:
 35
 10 mM each DATP, DCTP, DCTP, dTTP
 0.6 μl 15 units RNasin
 2.5 μl 40 mM Pyrophosphate
 1.5 μl 15 units reverse transcriptase
 Primary reaction (100 μl)
 40
 PCR Reactions:
 45
 10 μl 10X buffer from Perkin Elmer Cetus GeneAmp Kit
 1.25 μM each stock of dATP, dCTP, dGTP, and dTTP
 2 μl 50 PMoles L42.2
 2 μl first strand GSS9L cDNA
 0.5 μl 2.5 units AmpliTaq DNA polymerase
 67.5 μl Water
 50 Reaction conditions, 40 cycles of 94°C, 1°, 50°C, 2°, 30°, 2°, 72°C, 2°.

Prep scale secondary reaction:

5
 EXAMPLE 9
 Cloning and Sequencing of the VEGF A Monomer
 PCR Amplification, Cloning and Sequencing of P4238
 10
 Two degenerate oligonucleotides were synthesized in order to amplify the cDNA encoding the peptide sequences of VEGF A subunit between Lys C fragment L 42 and typtic fragment T38. These oligonucleotides were:
 15
 Cloning and Sequencing of the VEGF A Monomer
 PCR Amplification, Cloning and Sequencing of P4238
 20
 142.2 5. TTGTCGACCT[TC]ATGGA[TC]GT[N]TA[TC]CA 3.
 SEQ ID NO:3
 T383B
 [AG]CA 3. SEQ ID NO:4
 where N=ACGT
 25
 Poly A+ RNA was isolated from GS-9L cells using the Fast Track RNA isolation kit from Invitrogen and the protocol provided. First strand cDNA synthesis was performed as follows:
 1 μg of GS-9L RNA was annealed to 1 μg of adapter primer T117,
 2.5 μl 100 mM DTT
 2.5 μl 10 mM each DATP, DCTP, DCTP, dTTP
 0.6 μl 15 units RNasin
 2.5 μl 40 mM Pyrophosphate
 1.5 μl 15 units reverse transcriptase
 and the reaction was incubated at 42°C for 1 hour, then diluted to 1 ml in 10 mM Tris-HCl / mM EDTA,
 pH 7.5.
 30
 5. GACTCGAGCATCCTGACCA[AG]TC[N]GT[AG]TT[TCC]TT
 1 μg of GS-9L RNA was annealed to 1 μg of adapter primer T117,
 2.5 μl 10X buffer (500 mM Tris-HCl pH 8.3, 750 mM KCl, 100 mM MgCl₂, 5 mM spermidine)
 3.0 μl Water
 70°C for 5 min, followed by cooling to room temperature. To this reaction was added:
 35
 10 mM each DATP, DCTP, DCTP, dTTP
 0.6 μl 15 units RNasin
 2.5 μl 40 mM Pyrophosphate
 1.5 μl 15 units reverse transcriptase
 Primary reaction (100 μl)
 40
 PCR Reactions:
 45
 10 μl 10X buffer from Perkin Elmer Cetus GeneAmp Kit
 1.25 μM each stock of dATP, dCTP, dGTP, and dTTP
 2 μl 50 PMoles L42.2
 2 μl first strand GSS9L cDNA
 0.5 μl 2.5 units AmpliTaq DNA polymerase
 67.5 μl Water
 50 Reaction conditions, 40 cycles of 94°C, 1°, 50°C, 2°, 30°, 2°, 72°C, 2°.

5	Reaction conditions 94°C, 1'; 55°C, 2'; 72°C, 30 cycles.	The PCR product was concentrated by Centrifcon 3D spin columns, purified on a 1% agarose gel, and digested with restriction endonuclease SmaI. The SmaI fragment was then ligated into pET28b(+). The SmaI fragment was isolated from SmaI cut pET28b(+). The SmaI fragment was then used to transform E. coli XL-1 blue. Plasmid DNA was isolated from white transformants and sequenced by the dideoxy chain termination method.
10	PCR Amplification, Cloning and Sequencing of PW-3	PCR reactions: Primary reaction 100 µl 10 µl 10X buffer from Peftein Genemamp Kit 18 µl 1.25 mM each stock of dATP, dCTP, dGTP, and dTTP 0.35 µl first strand GS-9L DNA 2 µl 50 PMoles oligo 289 0.5 µl 2.5 units AmpliTaq DNA polymerase 67.15µl Water Reaction conditions 94°C, 1'; 58°C, 2'; 72°C, 10 cycles then add 50 PMoles A17, then 1 cycle of 94°C, 1'; 58°C, 2'; 72°C, 40, followed by 40 cycles 94°C, 1'; 58°C, 2'; 72°C, 2'. Reaction conditions 94°C, 1'; 58°C, 2'; 72°C, 10 cycles then add 50 PMoles A17, then 1 cycle of 94°C, 1'; 58°C, 2'; 72°C, 40, followed by 40 cycles 94°C, 1'; 58°C, 2'; 72°C, 2'. Based on the sequence obtained from the p423B clones, two specific PCR primers were synthesized; oligo 289 5', TTGTCTGACATCAGAGAGAAAGC 3', SEQ ID NO:6 and oligo 289 5', TTGTCTGACAGC 3', SEQ ID NO:7. These primers were used in combination with oligo A17 5', GACCTCGAGTCATCG 3', SEQ ID NO:8 to amplify the cDNA encoding the COOH terminus of VEGF A subunit using the 3', RACE technique described by Froehman et al., PNAS 85: 8998-9002 (1988).
20	PCR Amplification, Cloning and Sequencing of PW-3	PCR reactions: Primary reaction 100 µl 10 µl 10X buffer from Peftein Genemamp Kit 18 µl 1.25 mM each stock of dATP, dCTP, dGTP, and dTTP 0.35 µl first strand GS-9L DNA 2 µl 50 PMoles oligo 289 0.5 µl 2.5 units AmpliTaq DNA polymerase 67.15µl Water Reaction conditions 94°C, 1'; 58°C, 2'; 72°C, 10 cycles then add 50 PMoles A17, then 1 cycle of 94°C, 1'; 58°C, 2'; 72°C, 40, followed by 40 cycles 94°C, 1'; 58°C, 2'; 72°C, 2'. Reaction conditions 94°C, 1'; 58°C, 2'; 72°C, 10 cycles then add 50 PMoles A17, then 1 cycle of 94°C, 1'; 58°C, 2'; 72°C, 40, followed by 40 cycles 94°C, 1'; 58°C, 2'; 72°C, 2'. Based on the sequence obtained from the p423B clones, two specific PCR primers were synthesized; oligo 289 5', TTGTCTGACATCAGAGAGAAAGC 3', SEQ ID NO:6 and oligo 289 5', TTGTCTGACAGC 3', SEQ ID NO:7. These primers were used in combination with oligo A17 5', GACCTCGAGTCATCG 3', SEQ ID NO:8 to amplify the cDNA encoding the COOH terminus of VEGF A subunit using the 3', RACE technique described by Froehman et al., PNAS 85: 8998-9002 (1988).
25	PCR Amplification, Cloning and Sequencing of PW-3	Prep Scale secondary reaction: 60 µl 10X buffer 108 µl 1.25 mM each stock of dATP, dCTP, dGTP, and dTTP 24 µl primary PCR reaction 12 µl 300 PMoles oligo 307 12 µl 300 PMoles oligo A17 3 µl 15 units AmpliTaq DNA polymerase 381µl Water Reaction conditions 94°C, 1'; 58°C, 2'; 72°C, 2'; 30 cycles. The PCR product was then digested with restriction endonuclease SmaI. The SmaI fragment was then ligated into pET28b(+). The ligation mix was used to transform E. coli XL-1 blue. Plasmid DNA was isolated from white transformants and sequenced by the dideoxy chain termination method.
30	PCR Amplification, Cloning and Sequencing of p5-15	40 The PCR product was purified on a 1% agarose gel and digested with restriction endonuclease SmaI. The SmaI fragment was then ligated into pET28b(+). The ligation mix was used to transform E. coli XL-1 blue. Plasmid DNA was isolated from white transformants and sequenced by the dideoxy chain termination method.
35	PCR Amplification, Cloning and Sequencing of p5-15	45 Based on the sequence of p423B clones, two specific PCR primers were synthesized; oligo 113 5', TTGTCTGACATCAGAGAGAAAGC 3', SEQ ID NO:9 and oligo 74 5', CTTCACTGGAGCACACAGGAGCTTGAAG 3', SEQ ID NO:10.
40	PCR Amplification, Cloning and Sequencing of p5-15	50 These primers were used in combination with oligo A17 5', GACTCGAGTCAGACATCG 3', SEQ ID NO:8 to amplify the cDNA encoding the COOH terminus of VEGF A subunit using the 3', RACE technique described by Froehman et al., PNAS 85: 8998-9002 (1988).
45	PCR Amplification, Cloning and Sequencing of p5-15	55 One µg of GS9L RNA was annealed to 1 µg of oligo 151 by incubating in a volume of 6 µl at 70°C for 5', followed by cooling to room temperature. To this reaction was added:

1.5 μ l	10X buffer (500mM Tris-HCl, pH 8.3, 750 mM KCl, 100 mM MgCl ₂ , 5 mM spermidine)	10	PCR Reactions:
2.5 μ l	10 mM DTT	25	Primary reaction (50 μ l)
0.6 μ l	25 units RNasin	5	The reaction was incubated at 42°C for 1 hour. Excess oligo 151 was removed by Centrifugation 100 spin columns and the 5' end of the cDNA was tallied by the addition of dATP, DCTP, dGTP, and dTTP. The reaction was diluted to a final volume of 150 μ l in 10 mM Tris-HCl, 1 mM EDTA, pH 7.5.
5 μ l	10X buffer from Perkin Elmer Cetus GeneAmp Kit	15	Primarily reverse transcriptase
8 μ l	1.25 mM each stock of dATP, DCTP, dGTP, and dTTP	20	25 PMoles oligo 113
5 μ l	first strand GS-9L cDNA prime with oligo 151 and tallied	25	1 μ l
1 μ l	25 PMoles oligo A17	30	1 μ l
1 μ l	25 PMoles oligo T117	35	12 μ l
1 μ l	10 PMoles oligo A17	40	3 μ l
0.25 μ l	1.25 units AmpliTaq DNA polymerase	45	411 μ l water
28.75 μ l	water	50	Reactions conditions 94°C, 1'; 55°C, 2'; 72°C, 30 cycles.
20	Prep scale secondary reaction:		
5	Cloning and sequencing of alternative forms of VEGF A cDNA		
40	Based on the sequence obtained from the pS-15 and PW-3 clones, two specific PCR primers were synthesized: oligo 5', C 5, TTGTCGACCACTGAGCTCTGC 3', SEQ ID NO:12 and oligo 181 5', TTGTCGACCGTGAGAGTCCTAGTC 3', SEQ ID NO:13. These primers were used together to amplify multiple cDNAs encoding alternative forms of the VEGF A subunit.		
45	Preparative PCR Reaction:		
50	1.25M each stock of dATP, DCTP, dGTP, and dTTP		
50 μ l	10X buffer	55	50 μ l first strand GS-9L cDNA
10 μ l	300PMoles oligo 5'C	60	10 μ l 300PMoles oligo 181
10 μ l	15 units AmpliTaq DNA polymerase	65	2.5 μ l 2.5 μ l water
337.5 μ l	water	70	337.5 μ l 1.25M each stock of dATP, DCTP, dGTP, and dTTP
75	The reaction conditions 94°C, 1'; 55°C, 2'; 72°C, 30 cycles.		

The PCR product was concentrated by Centrifugation 30 spin columns and digested with restriction endonuclease Xba I and $Hind$ III.

30	60 μ l	10X buffer	1.25M each stock of dATP, dCTP, dGTP, and dTTP	12 μ l	first strand 659L cDNA	12 μ l	500PMoles oligo YI	12 μ l	500PMoles oligo GC	12 μ l	15 units AmpliTaq DNA polymerase	3 μ l	water	405 μ l	Master mix
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rep scale reaction:

Reaction conditions, 40 cycles of 94°C, 1'; 50°C, 2'; 72°C, 2'.

5 μL	10X buffer from Perkin Elmer Cetus GeneAmp Kit	Primarily reaction (50μL)
8 μL	1.25 MMs each stock of dATP, dCTP, dGTP, and dTTP	
1 μL	first strand GS-9L cDNA	
1 μL	50 PMoles oligo YI	
1 μL	50 PMoles oligo GC	
0.25 μL	1.25 units AmpliTaq DNA polymerase	
	water	33.75 μL

PCR Reactions:

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Collapsing and scaffolding of the VEGF-B subunit

EXAMPLE 10

and sequenced by the diideoxy chain termination method. Three sets of clones were identified. Clone #12 encodes the 190 amino acid chain terminating at Asn¹⁴⁰. The 164 amino acid secreted form of VEGF A subunit is that amino acid sequence running continuously from Ala²⁷ to Arg¹⁹⁰. Clone #14 has a 135 base pair deletion between the second base of the Asn¹⁴⁰ codon and the third base of the Arg¹⁹⁰ codon. This clone thus encodes a 146 aa form of the VEGF A subunit with the deletion of the 16 amino acids secreted between the second and third base of the Asn¹⁴⁰ codon. This clone thus encodes the 214 amino acid form of the VEGF A subunit with the conversion of Asn¹⁴⁰ to Lys¹⁴⁰. Figure 6.

5	Based on the sequence obtained from the PYG clones, a specific PCR primer was synthesized; oligo HP	5. TTGGTCGACCAACCTAATGAGATGC 3'. SEQ ID NO:16.	GACTCGAGTGCAGCATCG 3'. SEQ ID NO:8 to amplify the DNA encoding the COOH terminus of the VEGF B subunit using the 3'. RACE technique described by Frahm <i>et al.</i> , NAs 85: 8998-9002 (1988).	72°C, 2'.	Reaction conditions 1 cycle of 94°C, 1'; 58°C, 2'; 72°C, 2'; followed by 40 cycles 94°C, 1'; 58°C, 2' and 72°C, 2'.
60	10X buffer from Perkin Elmer Cetus Gene Amp Kit	12 μl first strand 659L DNA	3 μl 300 PMoles oligo A17	405 μl water	PCR Amplification, Cloning and Sequencing of p5V2
96	1.25 mM each of dATP, DCTP, DGTP, DTTP	12 μl 300 PMoles oligo A17	15 units Ampliqa DNA polymerase	3 μl	The PCR product was concentrated by Centrifcon 30 spin columns, precipitated with ethanol and digested with DNase I.
12	10X buffer from Perkin Elmer Cetus Gene Amp Kit	12 μl 300 PMoles oligo A17	300 PMoles oligo A17	12 μl	The PCR product was concentrated by Centrifcon 30 spin columns, precipitated with ethanol and digested with DNase I.
405	72°C, 2'.	405 μl water	15 units Ampliqa DNA polymerase	3 μl	1.25 mM each of dATP, DCTP, DGTP, DTTP
30	Based on the sequence of PYG clones, two specific PCR primers were synthesized; oligoV-L	96 μl	1.25 mM each of dATP, DCTP, DGTP, DTTP	12 μl	first strand 659L DNA
35	5. TTGGTCGACCAACCTAATGAGATGC 3'. SEQ ID NO:16.	96 μl	300 PMoles oligo A17	12 μl	300 PMoles oligo A17
40	Based on the sequence of PYG clones, two specific PCR primers were synthesized; oligoV-L	96 μl	Water	3 μl	Water
45	5. GACTCGAGTGCAGCATCG 3'. SEQ ID NO:8 to amplify the DNA encoding the COOH terminus of the VEGF B subunit using the 3'. RACE technique described by Frahm <i>et al.</i> , NAs 85: 8998-9002 (1988).	96 μl	Water	12 μl	1.25 mM each of dATP, DCTP, DGTP, DTTP
50	5. GAGCACACGACATCG 3'. SEQ ID NO:18. These primers were used in combination with oligo A17	96 μl	Water	12 μl	300 PMoles oligo A17
55	Based on the sequence of PYG clones, two specific PCR primers were synthesized; oligoV-L	96 μl	Water	3 μl	1.25 mM each of dATP, DCTP, DGTP, DTTP
59	5. GACTCGAGTGCAGCATCG 3'. SEQ ID NO:8 to amplify the DNA encoding the COOH terminus of the VEGF B subunit using the 3'. RACE technique described by Frahm <i>et al.</i> , NAs 85: 8998-9002 (1988).	96 μl	Water	12 μl	300 PMoles oligo A17
60	10X buffer from Perkin Elmer Cetus Gene Amp Kit	96 μl	Water	3 μl	Water
65	Based on the sequence obtained from the PYG clones, a specific PCR primer was synthesized; oligo HP	96 μl	Water	12 μl	1.25 mM each of dATP, DCTP, DGTP, DTTP
70	5. TTGGTCGACCAACCTAATGAGATGC 3'. SEQ ID NO:16.	96 μl	Water	12 μl	300 PMoles oligo A17
75	Based on the sequence obtained from the PYG clones, a specific PCR primer was synthesized; oligo HP	96 μl	Water	3 μl	Water
80	5. GACTCGAGTGCAGCATCG 3'. SEQ ID NO:8 to amplify the DNA encoding the COOH terminus of the VEGF B subunit using the 3'. RACE technique described by Frahm <i>et al.</i> , NAs 85: 8998-9002 (1988).	96 μl	Water	12 μl	1.25 mM each of dATP, DCTP, DGTP, DTTP
85	5. TTGGTCGACCAACCTAATGAGATGC 3'. SEQ ID NO:16.	96 μl	Water	12 μl	300 PMoles oligo A17
90	Based on the sequence obtained from the PYG clones, a specific PCR primer was synthesized; oligo HP	96 μl	Water	3 μl	Water
95	5. TTGGTCGACCAACCTAATGAGATGC 3'. SEQ ID NO:16.	96 μl	Water	12 μl	1.25 mM each of dATP, DCTP, DGTP, DTTP
100	Based on the sequence obtained from the PYG clones, a specific PCR primer was synthesized; oligo HP	96 μl	Water	3 μl	Water

Preparative PCR reaction:

Based on the sequence obtained from the PYG clones, a specific PCR primer was synthesized; oligo HP 5' TTTGTGCACACCCCTAATGAGTGC 3' SEQ ID NO:16. This primer was used in combination with oligo A17' 5' GACTCGAGTCATCAGTGC 3' SEQ ID NO:8 to amplify the cDNA encoding the COOH terminus of the VEGF B subunit using the 3' RACE technique described by Froehman et al., PNAS 85: 8998-9002 (1988).

PCR Amplification, Cloning and Sequencing of p3V2

E. coli XL-1 blue. Plasmid DNA was isolated from Sall cut pGEM3Z(+). The ligation mix was used to ligate the released Sall fragment with the Sall fragment isolated from Sall cut pGEM3Z(+).

PCR Amplification, Cloning and Sequencing of PCV2 and PCV2.1	
Based on the sequences of the P3V2 and P5CV2 clones, two specific PCR primers were synthesized; oligo 5CV2.1.	Based on the sequences of the P3V2 and P5CV2 clones, two specific PCR primers were synthesized; oligo 5CV2.1.
5. TTGGTCAAC[N][N]GCAAGTCCTACAGCTG 3'. SEq ID No:19 and oligo 3'CV2 5'.	These primers were used together to amplify the DNA encoding the VEGF B subunit.
TTGGTCAAC[N][N]GCAAGTCCTACAGCTG 3'. SEq ID No:20 and oligo 3'CV2 5'.	These primers were used together to amplify the DNA encoding the VEGF B subunit.
40 μ l 10X buffer	Preparative PCR Reaction:
64 μ l 1.25 M each dATP, dTTP, dGTP, dCTP	
8 μ l first strand GS-9L cDNA	
8 μ l 200 PMoles 5'CV2.1	
8 μ l 200 PMoles 3'CV2	
2 μ l 10Units AmpliTaq DNA Polymerase	
270 μ l water	
Reaction conditions: 94°C, 1'; 58°C, 2'; 72°C, 2'; 40 cycles.	
The PCR product was extracted with phenol/chloroform, concentrated by Centrificon 30 spin columns, precipitated by ethanol, and digested with restriction endonuclease SalI, and ligated into SalI cut PGEM3Z(+). The ligation mix was used to transform E. coli XL-1 blue. Plasmid DNA was isolated from white transformants and sequenced by the dideoxy chain termination method.	
158 amino acid sequence and the other encoded a 138 amino acid sequence, see Figures 7 and 8.	
CDNA Cloning of VEGF B Subunit	

1 μL	25 PMoles oligo VL	25 PMoles oligo VL	1 μL	25 PMoles oligo A17	25 PMoles oligo A17	1 μL	10 PMoles oligo TA17	10 PMoles oligo TA17	1 μL	1.25 units AmpliTaq DNA polymerase	1.25 μL	water	88.75 μL
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5 Second Strand Synthesis:
 5.5 μl 40M Nappi
 13.5 μl (55units) reverse transcriptase
 Incubate at 42°C 60'.
 50 μl first strand reaction
 25 μl 10X buffer (500 mM Tris-HCl, pH 7.2, 850 mM KCl, 30 mM MgCl₂, 1mg/ml BSA, 100 mM (NH₄)₂SO₄,
 7.5 μl 100 mM DTT
 25 μl 1mM NAD
 6.5 μl (65units) E. coli DNA Polymerase I
 2.5 μl (2.5units) E. coli DNA Ligase
 2.5 μl (2 units) E. coli RNase H
 135 μl Water
 15 Incuabte at 14°C for 2h and then incubate 70°C for 10'. Add 1μl (10 units) T4 DNA Polymerase, incubate
 20 DNA library Construction
 The above DNA was ligated into EcoR1/XbaI digested LambdaGEM-4 (Promega Biotechemicals) after the addition of EcoR1 linkers and digestion with EcoR1 and XbaI. A DNA library was amplified from ~50,000 independent clones.
 25 Isolation of Rat VEGF B cDNA Clone
 The above cDNA library was screened by plaque hybridization using CV2 as a probe. Hybridization con-
 ditions were as follows:
 30 0.15 mg/ml salmon sperm DNA hybridize overnight at 42°C.
 SDS at 50C for 30'. Positive clones were identified by autoradiography.
 Filters were washed 3 times in 2XSSC, 0.1% SDS at room temperature for 5', then 1 time in 1XSSC, 0.1%
 35 SDS at 50C for 30'. Positive clones were identified by autoradiography.
 The DNA from phage #202 was digested with restriction enzyme XbaI blue. Plasmid DNA was isolated from white transformants and sequenced by the diideoxy chain termination method. The cDNA was
 40 isolated from XbaI digested PGEM3zf(+). The ligation mix was used to transform E. coli XL-1 blue. Plasmid DNA was isolated from white transformants and sequenced by the diideoxy chain termination method. The cDNA was
 45 isolated from XbaI digested PGEM3zf(+). The entire nucleotide sequence of the signal peptide is shown in Fig. 7. The secreted protein starts at Ala₂₄ and continues to Arg₃₃. The entire nucleotide and amino acid sequence of the 158 amino acid form is shown in Figures 8. The secreted protein starts at Ala₂₄ and continues to Leu₁₅₈.

EXAMPLE 11

45 PCR Amplification, Cloning and Sequencing of PSEMI
 Cloning and sequencing VEGF C Subunit

50 The entire nucleotide and amino acid sequence of the signal peptide are shown in Figures 7 and 8.

The entire nucleotide and amino acid sequence of the signal peptide is shown in Figures 7 and 8. The secreted protein starts at Ala₂₄ and continues to Arg₃₃. The entire nucleotide and amino acid sequence of the 158 amino acid form is shown in Figures 8. The secreted protein starts at Ala₂₄ and continues to Leu₁₅₈.

55 Two degenerate oligonucleotides were synthesized based on the sequence of rat VEGF B monomer in order to amplify VEGF cDNAs from the human medulliblastoma line TE-671, ATCC HTB (McAllister et al., Int.

60 J. Cancer 20:206-212 [1977]. These oligonucleotides were: FS 5'TTGTCTGACA ATC AGA GAA (N) GT CAT (CT) GC 3' EM, 5' TTGTCGACA CTG AGA GAA (N) GT CAT (CT) GC 3' SEQ ID NO:21

65 Poly A+ RNA was isolated from TE-671 cells using the Fast Track RNA isolation kit from Invitrogen and the protocol provided. First strand cDNA synthesis was performed as follows using the cDNA Cycle kit from Invitrogen:

70 where N=AGCT
 SEQ ID NO:22

The Sall fragment was then ligated into Sall cut PGEM3Z(+), and the ligation mix used to transform E. coli. The PCR product was purified on a Qiagene tip 5 column, then digested with restriction endonuclease Sall.

Reaction conditions: 40 cycles of 94°C, 1'; 2' ramp to 58°C; 2' at 58°C; 2' at 72°C.

57.5 μl	water	55
0.5 μl	2.5 units AmpliTaq DNA polymerase	
2 μl	20 pmoles TATA primer	
2 μl	50 pmoles A17 primer	
10 μl	first strand TE-671 cDNA primed with V.E.	50
16 μl	1.25M each of dATP, dCTP, dGTP, TTP	49
10 μl	10X buffer from Perkin Elmer Getus GeneAmp kit	48
5, RACE PCR 5 X 100μl		

The reaction was incubated for 60' at 42°C, then 3' at 95°C, placed on ice, then an additional 1.25μl reverse transcriptase was added and the reaction incubated an additional 60' at 42°C. Excess oligo V.E. was removed by a Centrifcon 100 spin column and the 5' end of the cDNA was tallied by the addition of dATP and terminal transferase. The tailed cDNA was diluted to a final volume of 200 μl in 10MM Tris-HCl, 1MM EDTA, pH 7.5.

0.5 μl	AMV reverse transcriptase (Promega) 10units	40
2.5 μl	25 mM DNTPs	
10 μl	5X RT buffer	
2.5 μl	RNAse inhibitor	
1.0 μl	1.0 μg Primer V.E.	35
5 μl	0.7 M Mercaptoethanol	
20-25 μl	water	
1.0 μl	1 μg of TE-671 polyA+ RNA	
Invitrogen and the V.E. primer		
Poly A+ RNA was isolated from TE-671 cells using the Fast Track RNA isolation kit from Invitrogen and the protocol provided. First strand cDNA synthesis was performed as follows using the cDNA Cycle kit from (1988).		30

5-GACTCGAGTGCACATCG 3', SEQ ID NO:3 to amplify the cDNA encoding the amino terminus of the VEGF C subunit using the 5', RACE technique described by Froehman et al., PNAS 85: 8998-9002

oligo A17, 5', GACTCAGATGCCACATCG 3', SEQ ID NO:8, and oligo TATA 25'. Based on the sequence obtained from the PFSEM clone, two specific PCR primers were synthesized:

20 PCR Amplification, Cloning and Sequencing of p5.16

XL-1 blue. Plasmid DNA was isolated from white transformants and sequenced by the dideoxy chain termination method. The Sall fragment was then ligated into Sall cut PGEM3Z(+), and the ligation mix used to transform E. coli.

The PCR product was purified on a Qiagene tip 5 column, then digested with restriction endonuclease Sall.

Reaction conditions: 40 cycles of 94°C, 1'; 2' at 58°C; 3' at 72°C.

123 μl	water	20
1.0 μl	5 units AmpliTaq DNA polymerase	
2 μl	50 pmoles A17 primer	
2 μl	50 pmoles LH primer	10
20 μl	first strand TE-671 cDNA primed with TATA	
32 μl	1.25M each of dATP, dCTP, dGTP, TTP	
20 μl	10X buffer from Perkin Elmer Getus GeneAmp kit	
3, RACE PCR		

The reaction was incubated for 60' at 42°C, then 3' at 95°C, placed on ice, then an additional 1.25μl reverse transcriptase was added and the reaction incubated an additional 60' at 42°C.

1.25 μl reverse transcriptase 12.5 units

SEQ ID NO:29
170
Pto Arg Arg
COC CGG AGG TAA

165
160
Gln Arg Pto Thr Asp Cys His Leu Cys Gly Asp Ala Val
CAG AGA CCC ACA GAC TGC CAC CTG TGC GCC GAT GCT GTT

The PCR product was purified on a Qiagene tip 5 column, then digested with restriction endonuclease Sall. The Sall fragment was then ligated into Sall cut pGEM3Z(+), and the ligation mix used to transform E. coli XL-1 blue. Plasmid DNA was isolated from Sall cut pGEM3Z(+), and the plasmid used to transform E. coli XL-1 blue. In the sequences of clones pHVC16 and pHVC2 base 463 (Fig. 9) was changed from a T to a C eliminating the translatable stop codon following amino acid 154; this results in the addition of 16 amino acids following amino acid Lys 154. The nucleotide sequence and the deduced amino acid sequence of this addition is:

155
Arg Arg Pto Thr Asp Cys His Leu Cys Gly Asp Ala Val

SEQUENCE LISTING
 (2) INFORMATION FOR SEQ ID NO:1:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 14 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: Not Applicable
 (D) TOPOLOGY: Linear

- (A) LENGTH: 14 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: Not Applicable
- (D) TOPOLOGY: Linear

- 50 (C) STRANDEDNESS: single
 (B) TYPE: nucleic acid
 (A) LENGTH: 28 base pairs
 (i) SEQUENCE CHARACTERISTICS:
 (2) INFORMATION FOR SEQ ID NO:6:
- 45 GACTCGAGTC GACATCGATT TTTTTTT TTTT SEQ ID NO:5:
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
 (D) TOPOLOGY: linear
 (C) STRANDEDNESS: single
 (B) TYPE: nucleic acid
 (A) LENGTH: 35 base pairs
 (i) SEQUENCE CHARACTERISTICS:
 (2) INFORMATION FOR SEQ ID NO:5:
- 40 CAGAGATTC GTGAGATC NGTTRYTTR CA 32 SEQ ID NO:4:
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
 (D) TOPOLOGY: linear
 (C) STRANDEDNESS: single
 (B) TYPE: nucleic acid
 (A) LENGTH: 32 base pairs
 (i) SEQUENCE CHARACTERISTICS:
 (2) INFORMATION FOR SEQ ID NO:4:
- 35 TTGGTCGACT TYATGGAGGT NTAYCA 26 SEQ ID NO:3:
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
 (D) TOPOLOGY: linear
 (C) STRANDEDNESS: single
 (B) TYPE: nucleic acid
 (A) LENGTH: 26 base pairs
 (i) SEQUENCE CHARACTERISTICS:
 (2) INFORMATION FOR SEQ ID NO:3:
- 30 Val Pro Phe Asn Glu Val
 Ala Leu Ser Ala Gly Asn Xaa Ser Thr Glu Met Glu Val
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
 (D) TOPOLOGY: linear
 (C) STRANDEDNESS: Not applicable
 (B) TYPE: amino acid
 (A) LENGTH: 19 amino acids
 (i) SEQUENCE CHARACTERISTICS:
 (2) INFORMATION FOR SEQ ID NO:2:
- 25 Val
 Ala Pro Thr Glu Glu Glu Lys Ala His Glu Val
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
 (D) TOPOLOGY: linear
 (C) STRANDEDNESS: single
 (B) TYPE: amino acid
 (A) LENGTH: 19 amino acids
 (i) SEQUENCE CHARACTERISTICS:
 (2) INFORMATION FOR SEQ ID NO:1:
- 20 Val Pro Phe Asn Glu Val
 Ala Leu Ser Ala Gly Asn Xaa Ser Thr Glu Met Glu Val
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
 (D) TOPOLOGY: linear
 (C) STRANDEDNESS: single
 (B) TYPE: nucleic acid
 (A) LENGTH: 32 base pairs
 (i) SEQUENCE CHARACTERISTICS:
 (2) INFORMATION FOR SEQ ID NO:5:
- 15 Val Pro Phe Asn Glu Val
 Ala Leu Ser Ala Gly Asn Xaa Ser Thr Glu Met Glu Val
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
 (D) TOPOLOGY: linear
 (C) STRANDEDNESS: single
 (B) TYPE: amino acid
 (A) LENGTH: 19 amino acids
 (i) SEQUENCE CHARACTERISTICS:
 (2) INFORMATION FOR SEQ ID NO:2:
- 10 Val
 Ala Pro Thr Glu Glu Glu Lys Ala His Glu Val
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
 (D) TOPOLOGY: linear
 (C) STRANDEDNESS: single
 (B) TYPE: amino acid
 (A) LENGTH: 19 amino acids
 (i) SEQUENCE CHARACTERISTICS:
 (2) INFORMATION FOR SEQ ID NO:1:
- 5 Val
 Ala Pro Thr Glu Glu Glu Lys Ala His Glu Val
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
 (D) TOPOLOGY: linear
 (C) STRANDEDNESS: single
 (B) TYPE: amino acid
 (A) LENGTH: 19 amino acids
 (i) SEQUENCE CHARACTERISTICS:
 (2) INFORMATION FOR SEQ ID NO:1:

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(x_i) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CTTCATCAT GCAGCACGC 18

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(x_i) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGGTCA TACCCGGA AGATGCC 28

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(x_i) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TGGTCA ACACAGGAC GCCTGAG 28

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(x_i) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GACTGAGTC GACATCG 17

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(x_i) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGGTCA GAAATCACG TAGGC 25

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(D) TOPOLOGY: linear

(x_i) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TGGTCA ACACAGGAC GCCTGAG 28

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xii) SEQUENCE DESCRIPTIION: SEQ ID NO:13:

TTCGCGACA ACCATGAGTC TTCTGC 26

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xii) SEQUENCE DESCRIPTIION: SEQ ID NO:14:

TTGGTCAAGC GTGAGAGTC TAGTTC 26

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xii) SEQUENCE DESCRIPTIION: SEQ ID NO:14:

TTGGTCGACA TAYATHGNC AYGARC 26

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xii) SEQUENCE DESCRIPTIION: SEQ ID NO:15:

TTGGTCGACT CRCTCTRCA RCANC 26

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xii) SEQUENCE DESCRIPTIION: SEQ ID NO:16:

TITGTCGACA CACCCATTAG AGTGC 27

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xii) SEQUENCE DESCRIPTIION: SEQ ID NO:16:

TITGTCGACA CACCCATTAG AGTGC 27

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- (2) INFORMATION FOR SEQ ID NO:18:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (x_i) SEQUENCE DESCRIPTION: SEQ ID NO:18:
- TTCGACCA ACAGCGACTC AGAAGG 26
- (2) INFORMATION FOR SEQ ID NO:17:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (x_i) SEQUENCE DESCRIPTION: SEQ ID NO:17:
- TTCGACCA CAGGATAT GAGCAC 27
- (2) INFORMATION FOR SEQ ID NO:18:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (x_i) SEQUENCE DESCRIPTION: SEQ ID NO:19:
- TTCGACCN NGCAGTCC AGCTG 25
- (2) INFORMATION FOR SEQ ID NO:19:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (x_i) SEQUENCE DESCRIPTION: SEQ ID NO:20:
- TTCGACCN NCTATAAAAT AGAGGG 26
- (2) INFORMATION FOR SEQ ID NO:20:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (x_i) SEQUENCE DESCRIPTION: SEQ ID NO:21:
- TTCGACCA TTCAAGCTT CTGAGT 27
- (2) INFORMATION FOR SEQ ID NO:22:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (x_i) SEQUENCE DESCRIPTION: SEQ ID NO:22:
- TTCGACCA CTGAGAGAN GTCTATYC 28

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(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:
TTTGTGACCA CTGCACTGTG TGCCGGTG 28

10

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:
TTTGTGACCA ACATTGGCCG TCTCCACC 28

20

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:
TTTGTGACCA ATCGCCGCAG CAGCCGGT 28

30

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:
TTTGTGACT GGCTCTGGAC GTCTGAG

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(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:
TTTGTGACCA CTGAAGAGTG TGACGG 26

50

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 51 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

(D) TOPOLOGY: linear
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

5 CAG AGA CCC ACA GAC TGC CAC CTG TGC GGC GAT
 GCT GTT 39

CCC CGG AGG TAA
 51

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 16
 (B) TYPE: amino acids
 15 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Gln Arg Pro Thr Asp Cys His Leu Cys Gly Asp
 20 Ala Val 5 10

Pro Arg Arg
 15

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 577 base pairs
 (B) TYPE: nucleic acid
 30 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

AACC 4
 35 ATG AAC TTT CTG CTC TCT TGG GTG CAC TGG ACC CTG GCT TTA CTG 49
 Met Asn Phe Leu Leu Ser Trp Val His Trp Thr Leu Ala Leu Leu
 5 10 15

CTG TAC CTC CAC CAT GCC AAG TGG TCC CAG GCT GCA CCC ACG ACA 94
 Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Thr Thr
 20 25 30

GAA GGG GAG CAG AAA GCC CAT GAA GTG GTG AAG TTC ATG GAC GTC 139
 Glu Gly Glu Gln Lys Ala His Glu Val Val Lys Phe Met Asp Val
 35 40 40

45 TAC CAG CGC AGC TAT TGC CGT CCG ATT GAG ACC CTG GTG GAC ATC 184
 Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile
 50 55 60

TTC CAG GAG TAC CCC GAT GAG ATA GAG TAT ATC TTC AAG CCG TCC 229
 50 Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser
 65 70 75

TGT GTG CCC CTA ATG CGG TGT GCG GGC TGC TGC AAT GAT GAA GAA GCC 274
 Cys Val Pro Leu Met Arg Cys Ala Gly Cys Cys Asn Asp Glu Ala
 5 80 85 90
 CTG GAG TGC GTG CCC ACG TCG GAG AGC AAC GTC ACT ATG CAG ATC 319
 Leu Glu Cys Val Pro Thr Ser Glu Ser Asn Val Thr Met Gln Ile
 95 100 105
 10 ATG CGG ATC AAA CCT CAC CAA AGC CAG CAC ATA GGA GAG ATG AGC 364
 Met Arg Ile Lys Pro His Gln Ser Gln His Ile Gly Glu Met Ser
 110 115 120
 TTC CTG CAG CAT AGC AGA TGT GAA TGC AGA CCA AAG AAA GAT AGA 409
 Phe Leu Gln His Ser Arg Cys Glu Cys Arg Pro Lys Lys Asp Arg
 15 125 130 135
 ACA AAG CCA GAA AAT CAC TGT GAG CCT TGT TCA GAG CGG AGA AAG 454
 Thr Lys Pro Glu Asn His Cys Glu Pro Cys Ser Glu Arg Arg Lys
 140 145 150
 20 CAT TTG TTT GTC CAA GAT CCG CAG ACG TGT AAA TGT TCC TGC AAA 499
 His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys
 155 160 165
 AAC ACA GAC TCG CGT TGC AAG GCG AGG CAG CTT GAG TTA AAC GAA 544
 25 Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu
 170 175 180
 CGT ACT TGC AGA TGT GAC AAG CCA AGG CGG TGA 577
 Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
 30 185 190
 (2) INFORMATION FOR SEQ ID NO:31:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 190 amino acids
 (B) TYPE: amino acids
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:
 Met Asn Phe Leu Leu Ser Trp Val His Trp Thr Leu Ala Leu Leu
 40 5 10 15
 Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Thr Thr
 45 20 25 30
 Glu Gly Glu Gln Lys Ala His Glu Val Val Lys Phe Met Asp Val
 50 35 40 45
 Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile
 55 50 55 60
 55 Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser

	65	70	75
5	Cys Val Pro Leu Met Arg Cys Ala Gly Cys Cys Asn Asp Glu Ala 80	85	90
	Leu Glu Cys Val Pro Thr Ser Glu Ser Asn Val Thr Met Gln Ile 95	100	105
10	Met Arg Ile Lys Pro His Gln Ser Gln His Ile Gly Glu Met Ser 110	115	120
	Phe Leu Gln His Ser Arg Cys Glu Cys Arg Pro Lys Lys Asp Arg 125	130	135
15	Thr Lys Pro Glu Asn His Cys Glu Pro Cys Ser Glu Arg Arg Lys 140	145	150
	His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys 155	160	165
20	Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu 170	175	180
	Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg 185	190	
25	(2) INFORMATION FOR SEQ ID NO:32:		
	(i) SEQUENCE CHARACTERISTICS:		
	(A) LENGTH: 445 base pairs		
	(B) TYPE: nucleic acid		
30	(C) STRANDEDNESS: single		
	(D) TOPOLOGY: linear		
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:		
	ACCA		4
35	ATG AAC TTT CTG CTC TCT TGG GTG CAC TGG ACC CTG GCT TTA CTG Met Asn Phe Leu Leu Ser Trp Val His Trp Thr Leu Ala Leu Leu 5	49	
	5	10	15
40	CTG TAC CTC CAC CAT GCC AAG TGG TCC CAG GCT GCA CCC ACG ACA Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Thr Thr 20	94	
	20	25	30
45	GAA GGG GAG CAG AAA GCC CAT GAA GTG GTG AAG TTC ATG GAC GTC Glu Gly Glu Gln Lys Ala His Glu Val Val Lys Phe Met Asp Val 35	139	
	35	40	45
	TAC CAG CGC AGC TAT TGC CGT CCG ATT GAG ACC CTG GTG GAC ATC Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile 50	184	
	50	55	60
50	TTC CAG GAG TAC CCC GAT GAG ATA GAG TAT ATC TTC AAG CCG TCC Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser	229	

	65	70	75
5	TGT GTG CCC CTA ATG CGG TGT GCG GGC TGC TGC AAT GAT GAA GAC 274 Cys Val Pro Leu Met Arg Cys Ala Gly Cys Cys Asn Asp Glu Ala 80 90	85	90
10	CTG GAG TGC GTG CCC ACG TCG GAG AGC AAC GTC ACT ATG CAG ATC 319 Leu Glu Cys Val Pro Thr Ser Glu Ser Asn Val Thr Met Gln Ile 95 100	95	105
	ATG CGG ATC AAA CCT CAC CAA AGC CAG CAC ATA GGA GAG ATG AGC 364 Met Arg Ile Lys Pro His Gln Ser Gln His Ile Gly Glu Met Ser 110 115	110	120
15	TTC CTG CAG CAT AGC AGA TGT GAA TGC AGA CCA AAG AAA GAT AGA 409 Phe Leu Gln His Ser Arg Cys Glu Cys Arg Pro Lys Lys Asp Arg 125 130	125	135
20	ACA AAG CCA GAA AAA TGT GAC AAG CCA AGG CGG TGA 445 Thr Lys Pro Glu Lys Cys Asp Lys Pro Arg Arg 140 145	140	145
(2) INFORMATION FOR SEQ ID NO:33:			
(i) SEQUENCE CHARACTERISTICS:			
25	(A) LENGTH: 146 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear		
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:			
30	Met Asn Phe Leu Leu Ser Trp Val His Trp Thr Leu Ala Leu Leu 5 10	5	15
	Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Thr Thr 20 25	20	30
35	Glu Gly Glu Gln Lys Ala His Glu Val Val Lys Phe Met Asp Val 35 40	35	45
	Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile 50 55	50	60
40	Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser 65 70	65	75
	Cys Val Pro Leu Met Arg Cys Ala Gly Cys Cys Asn Asp Glu Ala 80 85	80	90
45	Leu Glu Cys Val Pro Thr Ser Glu Ser Asn Val Thr Met Gln Ile 95 100	95	105
50	Met Arg Ile Lys Pro His Gln Ser Gln His Ile Gly Glu Met Ser 110 115	110	120

Phe Leu Gln His Ser Arg Cys Glu Cys Arg Pro Lys Lys Asp Arg
 125 130 135

5 Thr Lys Pro Glu Lys Cys Asp Lys Pro Arg Arg
 140 145

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 649 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

15

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AACC

ATG AAC TTT CTG CTC TCT TGG GTG CAC TGG ACC CTG GCT TTA CTG 49
 Met Asn Phe Leu Leu Ser Trp Val His Trp Thr Leu Ala Leu Leu
 20 5 10 15

CTG TAC CTC CAC CAT GCC AAG TGG TCC CAG GCT GCA CCC ACG ACA 94
 Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Thr Thr
 25 20 25 30

25

GAA GGG GAG CAG AAA GCC CAT GAA GTG GTG AAG TTC ATG GAC GTC 139
 Glu Gly Glu Gln Lys Ala His Glu Val Val Lys Phe Met Asp Val
 35 35 40 45

30

TAC CAG CGC AGC TAT TGC CGT CCG ATT GAG ACC CTG GTG GAC ATC 184
 Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile
 50 55 60

35

TTC CAG GAG TAC CCC GAT GAG ATA GAG TAT ATC TTC AAG CCG TCC 229
 Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser
 65 70 75

40

TGT GTG CCC CTA ATG CGG TGT GCG GGC TGC TGC AAT GAT GAA GCC 274
 Cys Val Pro Leu Met Arg Cys Ala Gly Cys Cys Asn Asp Glu Ala
 80 85 90

45

CTG GAG TGC GTG CCC ACG TCG GAG AGC AAC GTC ACT ATG CAG ATC 319
 Leu Glu Cys Val Pro Thr Ser Glu Ser Asn Val Thr Met Gln Ile
 95 100 105

ATG CGG ATC AAA CCT CAC CAA AGC CAG CAC ATA GGA GAG ATG AGC 364
 Met Arg Ile Lys Pro His Gln Ser Gln His Ile Gly Glu Met Ser
 110 115 120

50

TTC CTG CAG CAT AGC AGA TGT GAA TGC AGA CCA AAG AAA GAT AGA 409
 Phe Leu Gln His Ser Arg Cys Glu Cys Arg Pro Lys Lys Asp Arg
 125 130 135

ACA AAG CCA GAA AAA AAA TCA GTT CGA GGA AAG GGA AAG GGT CAA 454

Thr Lys Pro Glu Lys Lys Ser Val Arg Gly Lys Gly Lys Gly Gln
 140 145 150
 5 AAA CGA AAG CGC AAG AAA TCC CCG TTT AAA TCC TGG AGC GTT CAC 499
 Lys Arg Lys Arg Lys Lys Ser Arg Phe Lys Ser Trp Ser Val His
 155 160 165
 TGT GAG CCT TGT TCA GAG CGG AGA AAG CAT TTG TTT GTC CAA GAT 544
 10 Cys Glu Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp
 170 175 180
 CCG CAG ACG TGT AAA TGT TCC TGC AAA AAC ACA GAC TCG CGT TGC 569
 Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys
 185 190 195
 15 AAG GCG AGG CAG CTT GAG TTA AAC GAA CGT ACT TGC AGA TGT GAC 634
 Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp
 200 205 210
 AAG CCA AGG CGG TGA
 20 Lys Pro Arg Arg 649

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 214 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

30 Met Asn Phe Leu Leu Ser Trp Val His Trp Thr Leu Ala Leu Leu
 5 10 15
 Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Thr Thr
 20 25 30
 35 Glu Gly Glu Gln Lys Ala His Glu Val Val Lys Phe Met Asp Val
 35 40 45
 Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile
 50 55 60
 40 Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser
 65 70 75
 Cys Val Pro Leu Met Arg Cys Ala Gly Cys Cys Asn Asp Glu Ala
 80 85 90
 45 Leu Glu Cys Val Pro Thr Ser Glu Ser Asn Val Thr Met Gln Ile
 95 100 105
 Met Arg Ile Lys Pro His Gln Ser Gln His Ile Gly Glu Met Ser
 110 115 120

Phe Leu Gln His Ser Arg Cys Glu Cys Arg Pro Lys Lys Asp Arg
 125 130 135
 5 Thr Lys Pro Glu Lys Lys Ser Val Arg Gly Lys Gly Lys Gly Gln
 140 145 150
 Lys Arg Lys Arg Lys Lys Ser Arg Phe Lys Ser Trp Ser Val His
 155 160 165
 10 Cys Glu Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp
 170 175 180
 Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys
 185 190 195
 15 Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp
 200 205 210
 Lys Pro Arg Arg
 20 (2) INFORMATION FOR SEQ ID NO:36:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 417 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:
 ATG CTG GCC ATG AAG CTG TTC ACT TGC TTC TTG CAG GTC CTA GCT 45
 Met Leu Ala Met Lys Leu Phe Thr Cys Phe Leu Gln Val Leu Ala
 5 10 15
 30 GGG TTG GCT GTG CAC TCC CAG GGG GCC CTG TCT GCT GGG AAC AAC 90
 Gly Leu Ala Val His Ser Gln Gly Ala Leu Ser Ala Gly Asn Asn
 20 25 30
 35 TCA ACA GAA ATG GAA GTG GTG CCT TTC AAT GAA GTG TGG GGC CGC 135
 Ser Thr Glu Met Glu Val Val Pro Phe Asn Glu Val Trp Gly Arg
 35 40 45
 40 AGC TAC TGC CGG CCA ATG GAG AAG CTG GTG TAC ATT GCA GAT GAA 180
 Ser Tyr Cys Arg Pro Met Glu Lys Leu Val Tyr Ile Ala Asp Glu
 50 55 60
 CAC CCT AAT GAA GTG TCT CAT ATA TTC AGT CCG TCA TGT GTC CTT 225
 His Pro Asn Glu Val Ser His Ile Phe Ser Pro Ser Cys Val Leu
 65 70 75
 45 CTG AGT CGC TGT AGT GGC TGC TGT GGT GAC GAG GGT CTG CAC TGT 270
 Leu Ser Arg Cys Ser Gly Cys Cys Gly Asp Glu Gly Leu His Cys
 80 85 90
 50
 55

GTG GCG CTA AAG ACA GCC AAC ATC ACT ATG CAG ATC TTA AAG ATT 315
 Val Ala Leu Lys Thr Ala Asn Ile Thr Met Gln Ile Leu Lys Ile
 95 100 105

5 CCC CCC AAT CGG GAT CCA CAT TCC TAC GTG GAG ATG ACA TTC TCT 360
 Pro Pro Asn Arg Asp Pro His Ser Tyr Val Glu Met Thr Phe Ser
 110 115 120

10 CAG GAT GTA CTC TGC GAA TGC AGG CCT ATT CTG GAG ACG ACA AAG 405
 Gln Asp Val Leu Cys Glu Cys Arg Pro Ile Leu Glu Thr Thr Lys
 125 130 135

GCA GAA AGG TAA
 Ala Glu Arg 417

- 15 (2) INFORMATION FOR SEQ ID NO:37:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 138 amino acids
 (B) TYPE: amino acid
 20 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Met Leu Ala Met Lys Leu Phe Thr Cys Phe Leu Gln Val Leu Ala
 25 5 10 15

Gly Leu Ala Val His Ser Gln Gly Ala Leu Ser Ala Gly Asn Asn
 20 25 30

30 Ser Thr Glu Met Glu Val Val Pro Phe Asn Glu Val Trp Gly Arg
 35 40 45

Ser Tyr Cys Arg Pro Met Glu Lys Leu Val Tyr Ile Ala Asp Glu
 50 55 60

35 His Pro Asn Glu Val Ser His Ile Phe Ser Pro Ser Cys Val Leu
 65 70 75

Leu Ser Arg Cys Ser Gly Cys Cys Gly Asp Glu Gly Leu His Cys
 80 85 90

40 Val Ala Leu Lys Thr Ala Asn Ile Thr Met Gln Ile Leu Lys Ile
 95 100 105

Pro Pro Asn Arg Asp Pro His Ser Tyr Val Glu Met Thr Phe Ser
 110 115 120

45 Gln Asp Val Leu Cys Glu Cys Arg Pro Ile Leu Glu Thr Thr Lys
 125 130 135

Ala Glu Arg

- 50 (2) INFORMATION FOR SEQ ID NO:38:
 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 477 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

ATG CTG GCC ATG AAG CTG TTC ACT TGC TTC TTG CAG GTC CTA GCT 45
 Met Leu Ala Met Lys Leu Phe Thr Cys Phe Leu Gln Val Leu Ala
 5 10 15

10 GGG TTG GCT GTG CAC TCC CAG GGG GCC CTG TCT GCT GGG AAC AAC 90
 Gly Leu Ala Val His Ser Gln Gly Ala Leu Ser Ala Gly Asn Asn
 20 25 30

15 TCA ACA GAA ATG GAA GTG GTG CCT TTC AAT GAA GTG TGG GGC CGC 135
 Ser Thr Glu Met Glu Val Val Pro Phe Asn Glu Val Trp Gly Arg
 35 40 45

20 AGC TAC TGC CGG CCA ATG GAG AAG CTG GTG TAC ATT GCA GAT GAA 180
 Ser Tyr Cys Arg Pro Met Glu Lys Leu Val Tyr Ile Ala Asp Glu
 50 55 60

25 CAC CCT AAT GAA GTG TCT CAT ATA TTC AGT CCG TCA TGT GTC CTT 225
 His Pro Asn Glu Val Ser His Ile Phe Ser Pro Ser Cys Val Leu
 65 70 75

30 CTG AGT CGC TGT AGT GGC TGC TGT GGT GAC GAG GGT CTG CAC TGT 270
 Leu Ser Arg Cys Ser Gly Cys Gly Asp Glu Gly Leu His Cys
 80 85 90

35 GTG GCG CTA AAG ACA GCC AAC ATC ACT ATG CAG ATC TTA AAG ATT 315
 Val Ala Leu Lys Thr Ala Asn Ile Thr Met Gln Ile Leu Lys Ile
 95 100 105

40 CCC CCC AAT CGG GAT CCA CAT TCC TAC GTG GAG ATG ACA TTC TCT 360
 Pro Pro Asn Arg Asp Pro His Ser Tyr Val Glu Met Thr Phe Ser
 110 115 120

45 CAG GAT GTA CTC TGC GAA TGC AGG CCT ATT CTG GAG ACG ACA AAG 405
 Gln Asp Val Leu Cys Glu Cys Arg Pro Ile Leu Glu Thr Thr Lys
 125 130 135

50 GCA GAA AGG AGG AAA ACC AAG GGG AAG AGG AAG CAA AGC AAA ACC 450
 Ala Glu Arg Arg Lys Thr Lys Gly Lys Arg Lys Gln Ser Lys Thr
 140 145 150

477
 45 CCA CAG ACT GAG GAA CCC CAC CTG TGA
 Pro Gln Thr Glu Glu Pro His Leu
 155

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 158 amino acids

50

55

- (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

	Met Leu Ala Met Lys Leu Phe Thr Cys Phe Leu Gln Val Leu Ala		
	5	10	15
10	Gly Leu Ala Val His Ser Gln Gly Ala Leu Ser Ala Gly Asn Asn		
	20	25	30
	Ser Thr Glu Met Glu Val Val Pro Phe Asn Glu Val Trp Gly Arg		
	35	40	45
15	Ser Tyr Cys Arg Pro Met Glu Lys Leu Val Tyr Ile Ala Asp Glu		
	50	55	60
20	His Pro Asn Glu Val Ser His Ile Phe Ser Pro Ser Cys Val Leu		
	65	70	75
	Leu Ser Arg Cys Ser Gly Cys Cys Gly Asp Glu Gly Leu His Cys		
	80	85	90
25	Val Ala Leu Lys Thr Ala Asn Ile Thr Met Gln Ile Leu Lys Ile		
	95	100	105
	Pro Pro Asn Arg Asp Pro His Ser Tyr Val Glu Met Thr Phe Ser		
	110	115	120
30	Gln Asp Val Leu Cys Glu Cys Arg Pro Ile Leu Glu Thr Thr Lys		
	125	130	135
	Ala Glu Arg Arg Lys Thr Lys Gly Lys Arg Lys Gln Ser Lys Thr		
	140	145	150
35	Pro Gln Thr Glu Glu Pro His Leu		
	155		

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:

- 40 (A) LENGTH: 465 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

45	ATG CCG GTC ATG AGG CTG TTC CCT TGC TTC CTG CAG CTC CTG GCC	45	
	Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala		
	5	10	15

50

55

GGG CTG GCG CTG CCT GCT GTG CCC CCC CAG CAG TGG GCC TTG TCT 90
 Gly Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser
 20 25 30
 5 GCT GGG AAC GGC TCG TCA GAG GTG GAA GTG GTA CCC TTC CAG GAA 135
 Ala Gly Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu
 35 40 45
 10 GTG TGG GGC CGC AGC TAC TGC CGG GCG CTG GAG AGG CTG GTG GAC 180
 Val Trp Gly Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp
 50 55 60
 15 GTC GTG TCC GAG TAC CCC AGC GAG GTG GAG CAC ATG TTC AGC CCA 225
 Val Val Ser Glu Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro
 65 70 75
 20 TCC TGT GTC TCC CTG CTG CGC TGC ACC GGC TGC TGC GGC GAT GAG 270
 Ser Cys Val Ser Leu Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu
 80 85 90
 25 AAT CTG CAC TGT GTG CCG GTG GAG ACG GCC AAT GTC ACC ATG CAG 315
 Asn Leu His Cys Val Pro Val Glu Thr Ala Asn Val Thr Met Gln
 95 100 105
 30 CTC CTA AAG ATC CGT TCT GGG GAC CGG CCC TCC TAC GTG GAG CTG 360
 Leu Leu Lys Ile Arg Ser Gly Asp Arg Pro Ser Tyr Val Glu Leu
 110 115 120
 ACG TTC TCT CAG CAC GTT CGC TGC GAA TGC CGG CCT CTG CGG GAG 405
 Thr Phe Ser Gln His Val Arg Cys Glu Cys Arg Pro Leu Arg Glu
 125 130 135
 35 AAG ATG AAG CCG GAA AGG AGG AGA CCC AAG GGC AGG GGG AAG AGG 450
 Lys Met Lys Pro Glu Arg Arg Arg Pro Lys Gly Arg Gly Lys Arg
 140 145 150
 405
 AGG AGA GAG AAG TAG 465
 Arg Arg Glu Lys

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 154 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

45 Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala
5 10 15

Gly Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser
20 25 30

50

Ala Gly Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu
 35 40 45
 5 Val Trp Gly Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp
 50 55 60
 Val Val Ser Glu Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro
 65 70 75
 10 Ser Cys Val Ser Leu Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu
 80 85 90
 Asn Leu His Cys Val Pro Val Glu Thr Ala Asn Val Thr Met Gln
 95 100 105
 15 Leu Leu Lys Ile Arg Ser Gly Asp Arg Pro Ser Tyr Val Glu Leu
 110 115 120
 Thr Phe Ser Gln His Val Arg Cys Glu Cys Arg Pro Leu Arg Glu
 125 130 135
 20 Lys Met Lys Pro Glu Arg Arg Arg Pro Lys Gly Arg Gly Lys Arg
 140 145 150
 Arg Arg Glu Lys
 25

Claims

- 30 1. A purified and isolated DNA sequence encoding the C subunit of vascular endothelial cell growth factor.
 2. A purified and isolated vascular endothelial cell growth factor C subunit DNA sequence comprising:

35 ATG COG GTC ATG AGG CTG TTC CCT TGC TTC CTG CAG CTC CTG
 GCC GGG CTG CGC CTG CCT GCT GTG CCC CCC CAG CAG TGG GCC
 TTG TCT GCT GGG AAC AAC GGC TCG TCA GAG GTG GAA GTG GTA CCC
 TTC CAG GAA GTG TGG GGC CGC AGC TAC TGC CGG CGG CTG GAG
 AGG CTG GTG GAC GTC GTG TCC GAG TAC CCC AGC GAG GTG GAG
 CAC ATG TTC AGC CCA TCC TGT GTC TCC CTG CTG CGC TGC ACC
 GGC TGC TGC GGC GAT GAG AAT CTG CAC TGT GTG CGG GTG GAG
 40 ACG GCC AAT GTC ACC ATG CAG CTC CTA AAG ATC CGT TCT GGG
 GAC CGG CCC TCC TAC GTG GAG CTG ACG TTC TCT CAG CAC GTT
 CGC TGC GAA TGC CGG CCT CTG CGG GAG AAG ATG AAG CGG GAA
 45 AGG AGG AGA CCC AAG GGC AGG GGG AAG AGG AGG AGA GAG AAG
 TAG. SEQ ID NO:40

- 50 3. Vascular endothelial cell growth growth factor AC DNA comprising an A subunit DNA sequence and a C subunit DNA sequence.
 55 4. Vascular endothelial cell growth growth factor BC DNA comprising a B subunit DNA sequence and a C subunit DNA sequence.

5. A purified and isolated vascular endothelial cell growth factor C subunit DNA sequence comprising:
ATG CCG GTC ATG AGG CTG TTC CCT TGC TTC CTG CAG CTC CTG

5 GCC GGG CTG GCG CTG OCT GCT GTG CCG CCC CAG CAG TGG GCC
TTG TCT GCT GGG AAC GGC TCG TCA GAG GTG GAA GTG GTA CCC
10 TTC CAG GAA GTG TGG GGC CGC AGC TAC TGC CGG CGC CTG GAG
AGG CTG GTG GAC GTC GTG TCC GAG TAC CCG AGC GAG GTG GAG
CAC ATG TTC AGC CCA TCC TGT GTC TCC CTG CTG CGC TGC ACC
GGC TGC TGC GGC GAT GAG AAT CTG CAC TGT GTG CGG GTG GAG
AAG GCC AAT GTC ACC ATG CAG CTC CTA AAG ATC CGT TCT GGG
15 GAC CGG CCC TCC TAC GTG GAG CTG ACG TTC TCT CAG CAC GTT
CGC TGC GAA TGC CGG CCT CTG CGG GAG AAG ATG AAG CGG GAA
AGG AGG AGA CGC AAG GGC AGG GGG AAG AGG AGG AGA GAG AAG
20 CAG AGA CCC ACA GAC TGC CAC CTG TGC GGC GAT GCT GTT CCC
CGG AGG TAA. SEQ ID NOS:29 & 40

- 25 6. Vascular endothelial cell growth factor AC DNA comprising an A subunit DNA sequence selected from the group consisting of: a DNA sequence encoding an 189 amino acid form, a DNA sequence encoding an 165 amino acid form and a DNA sequence encoding a 121 amino acid form, with said A subunit DNA operably attached to a C subunit DNA sequence.
- 30 7. Vascular endothelial cell growth factor BC DNA comprising a B subunit DNA sequence selected from the group consisting of a DNA sequence encoding a 135 amino acid form; and a DNA sequence encoding a 115 amino acid form, with said B subunit DNA sequence operably attached to a C subunit DNA sequence.
- 35 8. Homodimeric vascular endothelial growth factor DNA comprising C subunit DNA sequences.
- 35 9. A vector containing the DNA sequence of any one of claims 3 to 8.
- 40 10. A host cell transformed by the vector of claim 9 containing the DNA sequence encoding vascular endothelial cell growth factor.
- 45 11. A process for the preparation of vascular endothelial cell growth factor comprising culturing the transformed host cell of claim 10 under conditions suitable for the expression of vascular endothelial cell growth factor and recovering vascular endothelial cell growth factor.
- 45 12. Vascular endothelial growth factor made by the process of claim 11.
- 45 13. Vascular endothelial cell growth factor AC comprising an A subunit amino acid sequence and a C subunit amino acid sequence.
- 50 14. Vascular endothelial cell growth factor BC comprising a B subunit amino acid sequence and a C subunit amino acid sequence.
- 50 15. Vascular endothelial cell growth factor CC comprising a C subunit amino acid sequence and a C subunit amino acid sequence.
- 55 16. A purified and isolated vascular endothelial cell growth factor C subunit amino acid sequence comprising:

Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu
 Ala Gly Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala
 5 Leu Ser Ala Gly Asn Gly Ser Ser Glu Val Glu Val Val Pro
 Phe Gln Glu Val Trp Gly Arg Ser Tyr Cys Arg Ala Leu Glu
 Arg Leu Val Asp Val Val Ser Glu Tyr Pro Ser Glu Val Glu
 His Met Phe Ser Pro Ser Cys Val Ser Leu Leu Arg Cys Thr
 10 Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro Val Glu
 Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly
 Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val
 15 Arg Cys Glu Cys Arg Pro Leu Arg Glu Lys Met Lys Pro Glu
 Arg Arg Arg Pro Lys Gly Arg Gly Lys Arg Arg Arg Glu Lys.
 SEQ ID NO:41

- 20 17. A purified and isolated vascular endothelial cell growth factor C subunit amino acid sequence comprising:

Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu
 25 Ala Gly Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala
 Leu Ser Ala Gly Asn Gly Ser Ser Glu Val Glu Val Val Pro
 Phe Gln Glu Val Trp Gly Arg Ser Tyr Cys Arg Ala Leu Glu
 Arg Leu Val Asp Val Val Ser Glu Tyr Pro Ser Glu Val Glu
 His Met Phe Ser Pro Ser Cys Val Ser Leu Leu Arg Cys Thr
 30 Gly Cys Cys Gly Asp Glu Asn Leu His Cys Val Pro Val Glu
 Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly
 Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val
 35 Arg Cys Glu Cys Arg Pro Leu Arg Glu Lys Met Lys Pro Glu
 Arg Arg Arg Pro Lys Gly Arg Gly Lys Arg Arg Arg Glu Lys
 Gln Arg Pro Thr Asp Cys His Leu Cys Gly Asp Ala Val Pro
 40 Arg Arg. SEQ ID NOS: 29 & 40

- 45 18. A tissue repairing pharmaceutical composition comprising a pharmaceutical carrier and an effective tissue repairing amount of the purified vascular endothelial growth factor of any one of claims 13 to 15,
- 46 19. The use of the vascular endothelial cell growth factor of any one of claims 13 to 15 for the manufacture of a medicament for promoting tissue repair.
- 50 20. The use of the vascular endothelial cell growth factor of any one of claims 13 to 15 for the manufacture of a medicament for stimulating vascular endothelial cell growth.

p5-15

1 10

A ACC ATG AAC TTT CTG CTC TCT TGG GTG CAC TGG ACC CTC GCT TTA CTG CTC TAC CTC CAC CAT
MET-ASN-PHE-LEU-LEU-SER-TRP-VAL-HIS-TRP-THR-LEU-ALA-LEU-LEU-TYR-LEU-HIS-HIS-

FIG. 1A

The diagram illustrates the p4238/p5-15 plasmid construct. The top horizontal line represents the plasmid backbone, with arrows indicating restriction sites: PstI (at position 1), EcoRI (at position 41), KpnI (at position 50), SphI (at position 60), and SalI (at position 69). The bottom horizontal line shows the sequence of the coding region, with arrows pointing upwards from the sequence. Key positions are labeled: T41, L42, T65, V40, and CB26. The sequence is as follows:

AAG TTC ATG GAC GTC TAC CAG CGC AGC TAT TGC CGT CCG ATT GAG ACC CTG GTG GAC ATC
 41
 50
 LYS-PHE-MET-ASP-VAL-TYR-GLN-ARG-SER-TYR-CYS-ARG-PRO-ILE-GLU-THR-LEU-VAL-ASP-ILE-
 T41
 L42
 T65
 V40
 CB26

FIG. 1 B

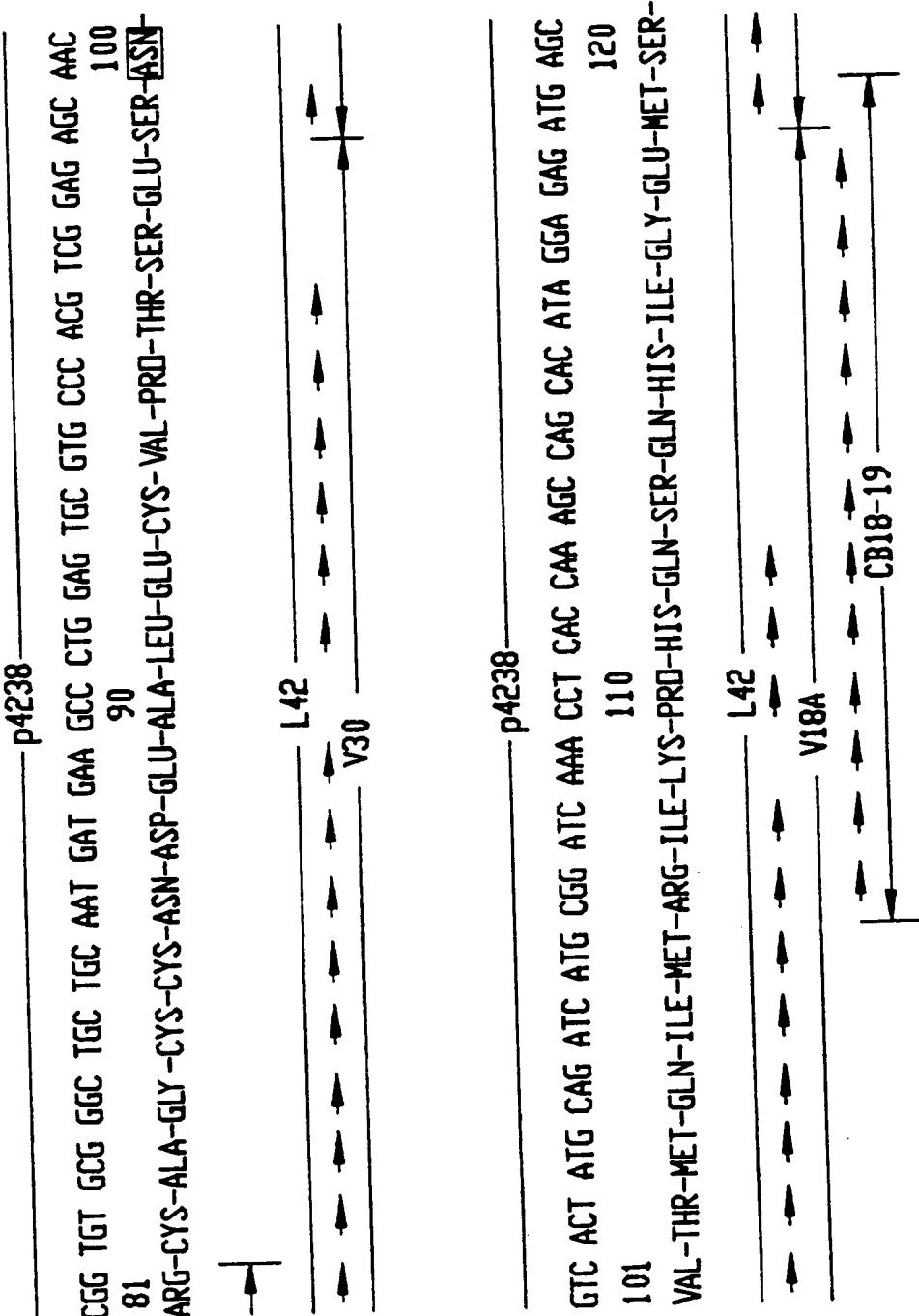


FIG. 1C

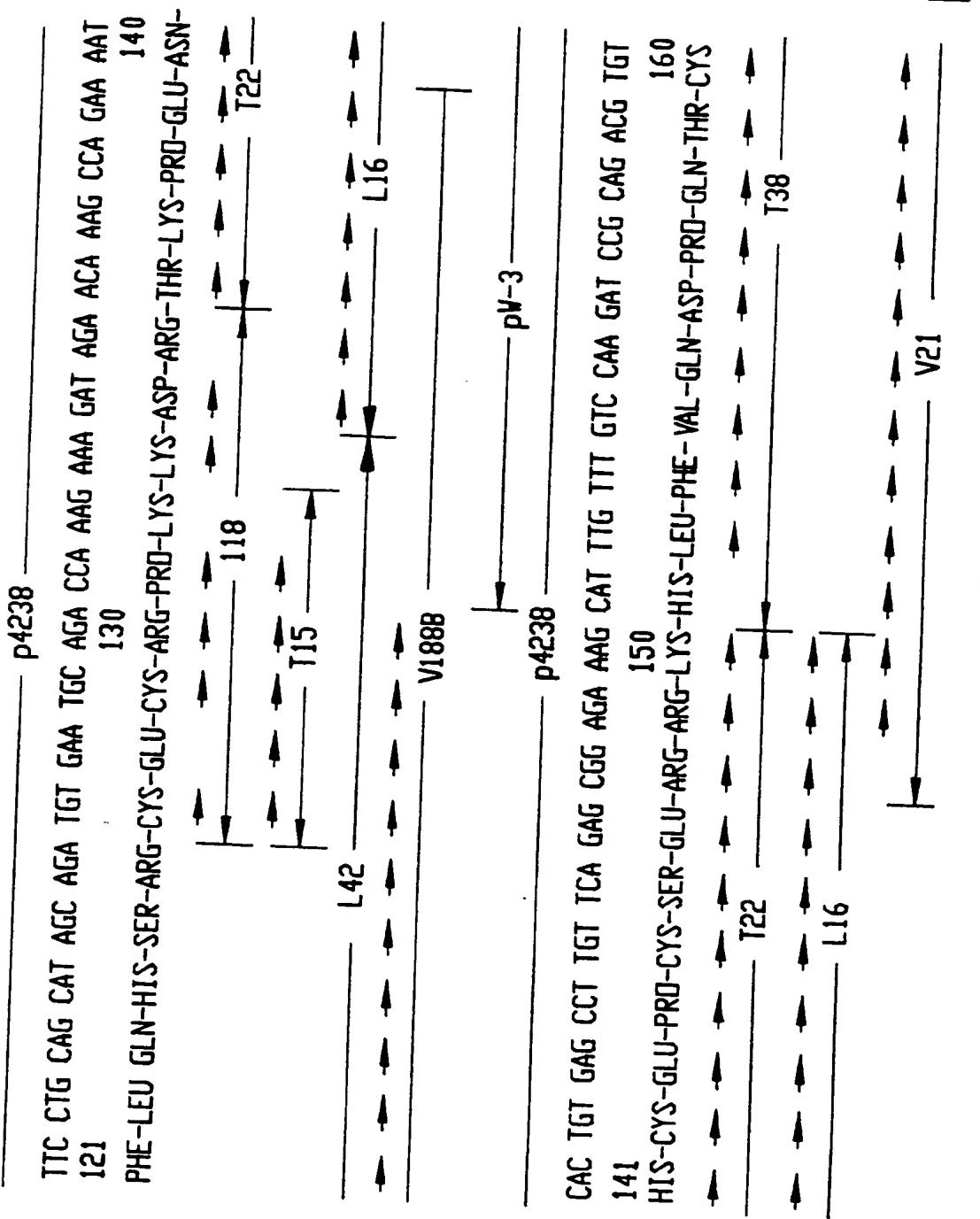


FIG. 1E

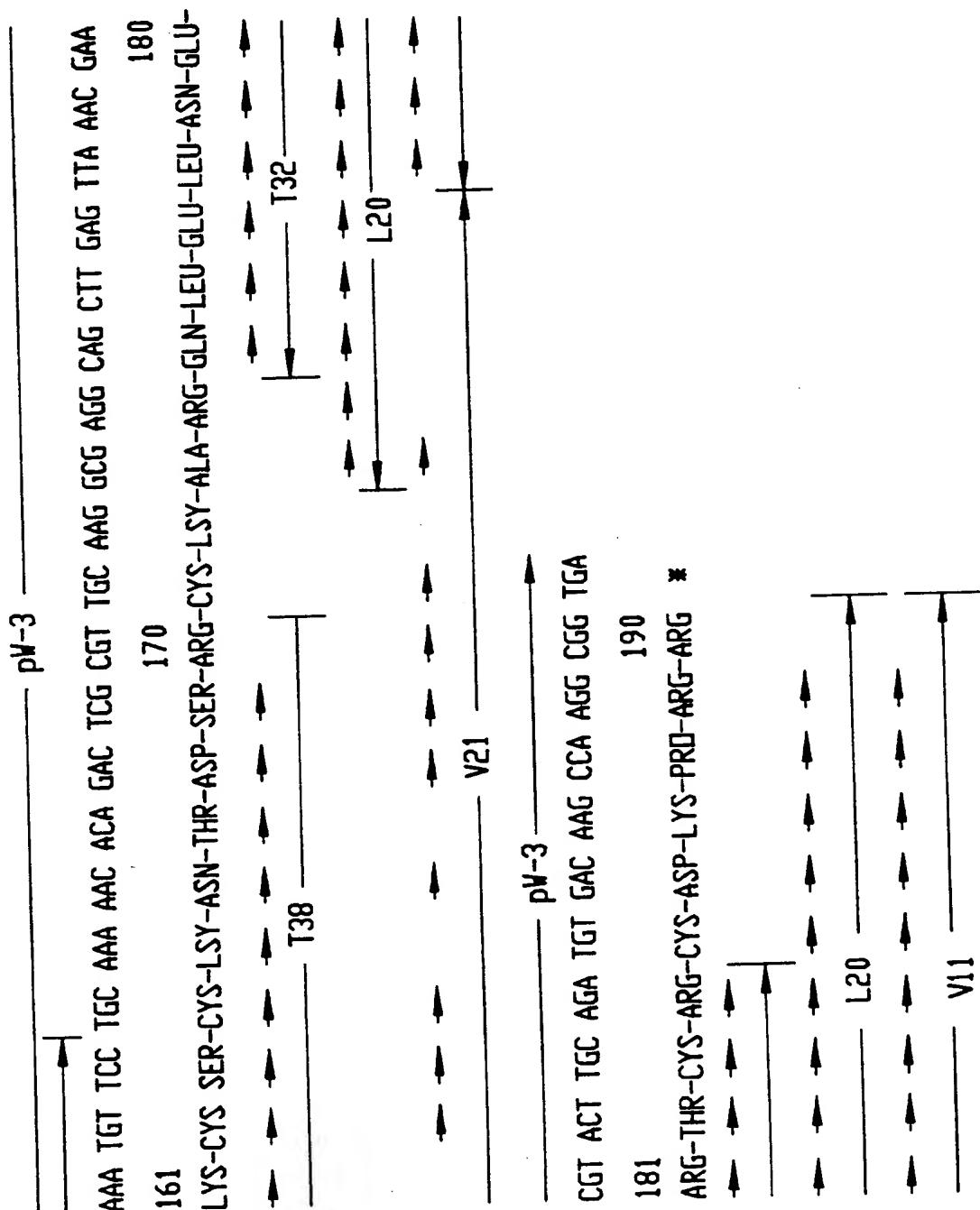


FIG. 2A

p4238

TTC CAG GAG TAC CCC GAT GAG ATA GAG TAT ATC TTC AAG CCG TCC TGT GTG CCC CTA ATG
 61 PHE-GLN-GLU-TYR-PRO-ASP-GLU-ILE-PHE-LYS-PRO-SER-CYS-VAL-PRO-LEU-MET-
 → → → → → L46

p4238-

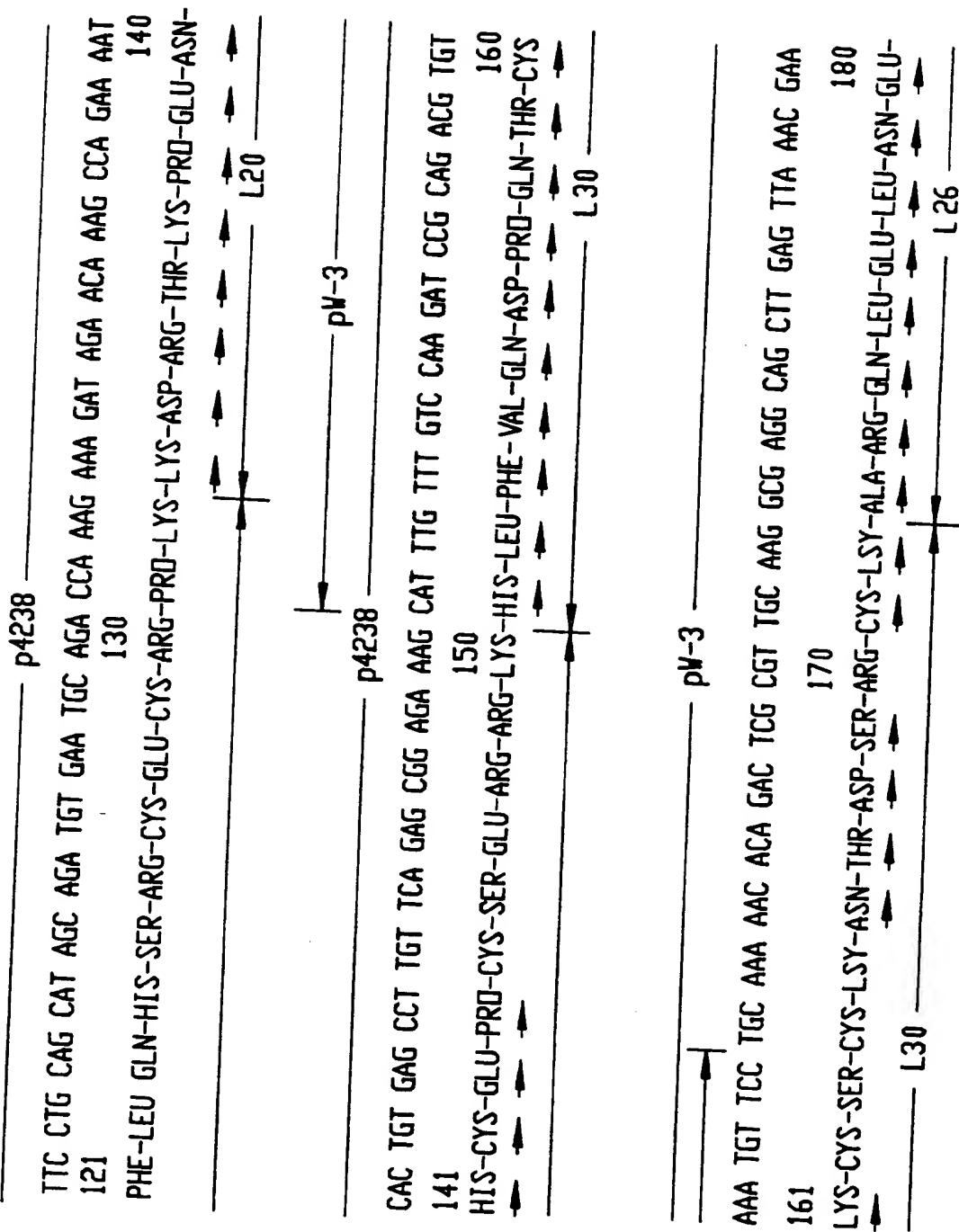
CGG TGT GCG GCC TGC TGC AAT GAT GAA GCC CTC GAG TGC CCC ACC TCG GAG AGC AAC
 81 ARG-CYS-ALA-GLY-CYS-CYS-ASN-ASP-GLU-ASP-GLU-ALA-LEU-GLU-CYS-VAL-PRO-THR-SER-GLU-SER-[ASN]-
 → → → → → L46

p4238

GTC ACT ATG CAG ATC ATG CGG ATC AAA CCT CAC CAA AGC CAG CAC ATA GGA GAG ATG AGC
 101 VAL-THR-MET-GLN-ILE-MET-ARG-ILE-LYS-PRO-HIS-GLN-SER-GLN-HIS-ILE-GLY-GLU-MET-SER-
 → → → → → L46

FIG. 2B

FIG. 2C



CGT ACT TGC AGA TGT GAC AAG CCA AGG CCG TGA
 181 AAG-THR-CYS-ARG-CYS-ASP-LYS-PRO-ARG-ARG *
 190
 pN-3

FIG. 2D

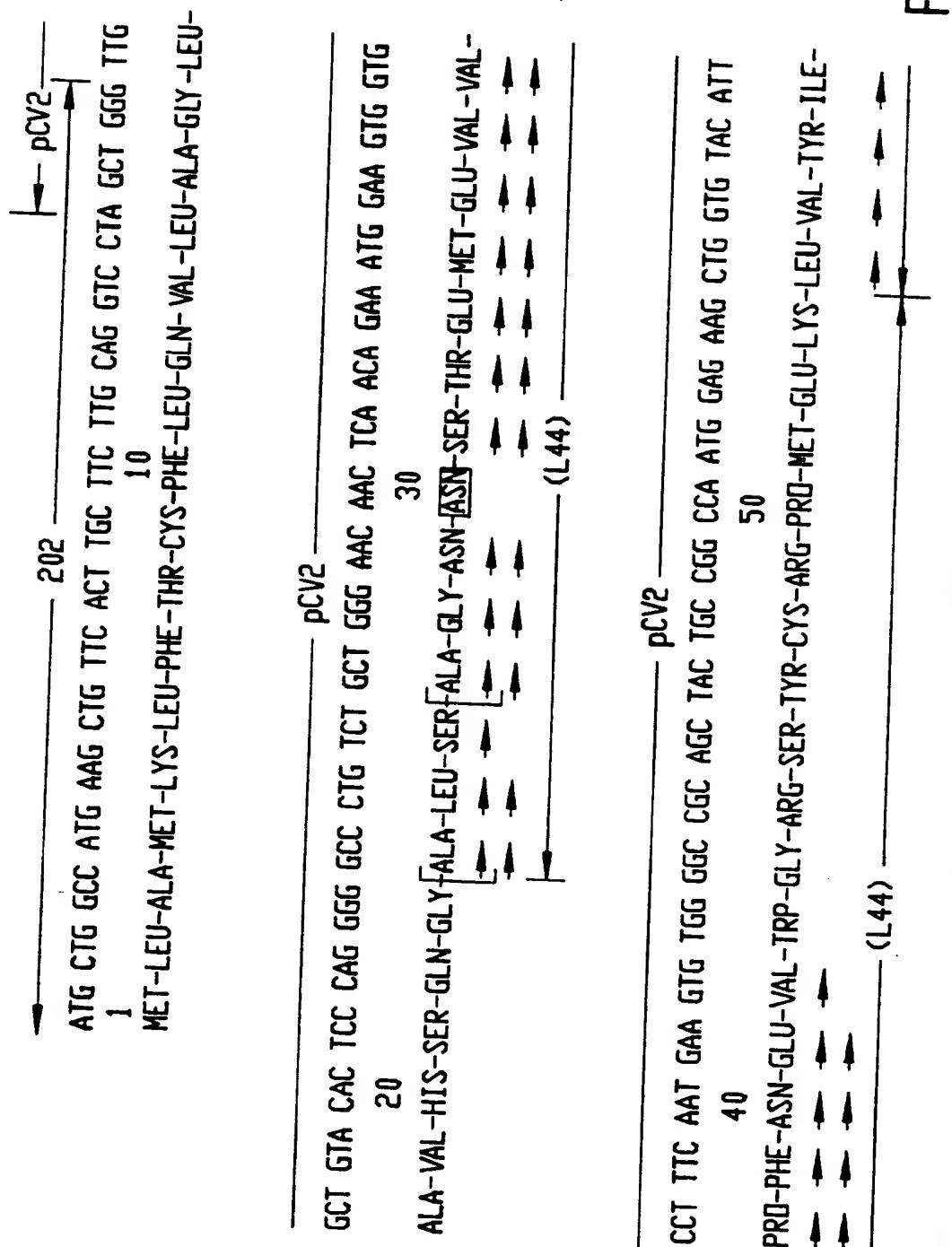


FIG.3A

pCV2 _____
 GCA GAT GAA CAC CCT AAT GAA GTG TCT CAT ATA TTC AGT CCG TCA TGT GTC CTT CTG AGT
 60
 ALA-ASP-GLU-HIS-PRO-ASN-GLU-VAL-SER-HIS-ILE-PHE-SER-PRO-SER-CYS-VAL-LEU-LEU-SER-
 70
 L50

pCV2 _____
 CCC TGT AGT GGC TGC TGT GGT GAC GAG GGT CTG CAC TGT GTG GCG CTA AAG ACA GCC AAC
 80
 90
 ARG-CYS-SER-GLY-CYS-CYS-GLY-ASP-GLU-GLY-LEU-HIS-CYS-VAL-ALA-LEU-LYS-THR-ALA-[ASN]
 100
 110
 120
 130
 140
 150

FIG. 3B

FIG. 3C

FIG. 4

AACC ATG AAC TTT CTG CTC TCT TGG GTG CAC TGG ACC CTG GCT TTA CTG 49
 Met Asn Phe Leu Leu Ser Trp Val His Trp Thr Leu Ala Leu Leu
 5 10 15

CTG TAC CTC CAC CAT GCC AAG TGG TOC CAG GCT GCA CCC ACG ACA 94
 Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Thr Thr
 20 25 30

GAA GGG GAG CAG AAA GCC CAT GAA GTG GTG AAG TTC ATG GAC GTC 139
 Glu Gly Glu Gln Lys Ala His Glu Val Val Lys Phe Met Asp Val
 35 40 45

TAC CAG CGC AGC TAT TGC CGT CCG ATT GAG ACC CTG GTG GAC ATC 184
 Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile
 50 55 60

TTC CAG GAG TAC CCC GAT GAG ATA GAG TAT ATC TTC AAG CCG TOC 229
 Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser
 65 70 75

TGT GTG CCC CTA ATG CGG TGT GCG GGC TGC TGC AAT GAT GAA GCC 274
 Cys Val Pro Leu Met Arg Cys Ala Gly Cys Cys Asn Asp Glu Ala
 80 85 90

CTG GAG TGC GTG CCC ACG TCG GAG AGC AAC GTC ACT ATG CAG ATC 319
 Leu Glu Cys Val Pro Thr Ser Glu Ser Asn Val Thr Met Gln Ile
 95 100 105

ATG CGG ATC AAA CCT CAC CAA AGC CAG CAC ATA GGA GAG ATG AGC 364
 Met Arg Ile Lys Pro His Gln Ser Gln His Ile Gly Glu Met Ser
 110 115 120

TTC CTG CAG CAT AGC AGA TGT GAA TGC AGA CCA AAG AAA GAT AGA 409
 Phe Leu Gln His Ser Arg Cys Glu Cys Arg Pro Lys Lys Asp Arg
 125 130 135

ACA AAG CCA GAA AAT CAC TGT GAG CCT TGT TCA GAG CGG AGA AAG 454
 Thr Lys Pro Glu Asn His Cys Glu Pro Cys Ser Glu Arg Arg Lys
 140 145 150

CAT TTG TTT GTC CAA GAT CGG CAG ACG TGT AAA TGT TOC TGC AAA 499
 His Leu Phe Val Gln Asp Pro Gln Thr Cys Lys Cys Ser Cys Lys
 155 160 165

AAC ACA GAC TOG CGT TGC AAG GCG AGG CAG CTT GAG TTA AAC GAA 544
 Asn Thr Asp Ser Arg Cys Lys Ala Arg Gln Leu Glu Leu Asn Glu
 170 175 180

CGT ACT TGC AGA TGT GAC AAG CCA AGG CGG TGA 577
 Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
 185 190

FIG. 5

AACC ATG AAC TTT CTG CTC TCT TGG GTG CAC TGG ACC CTG GCT TTA CTG	49	
Met Asn Phe Leu Leu Ser Trp Val His Trp Thr Leu Ala Leu Leu		
5	10	15
CTG TAC CTC CAC CAT GCC AAG TGG TCC CAG GCT GCA OOC ACG ACA	94	
Leu Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Thr Thr		
20	25	30
GAA GGG GAG CAG AAA GCC CAT GAA GTG GTG AAG TTC ATG GAC GTC	139	
Glu Gly Glu Gln Lys Ala His Glu Val Val Lys Phe Met Asp Val		
35	40	45
TAC CAG CGC AGC TAT TGC CGT CGG ATT GAG ACC CTG GTG GAC ATC	184	
Tyr Gln Arg Ser Tyr Cys Arg Pro Ile Glu Thr Leu Val Asp Ile		
50	55	60
TTC CAG GAG TAC CCC GAT GAG ATA GAG TAT ATC TTC AAG CCG TCC	229	
Phe Gln Glu Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser		
65	70	75
TGT GTG CCC CTA ATG CGG TGT GCG GGC TGC TGC AAT GAT GAA GGC	274	
Cys Val Pro Leu Met Arg Cys Ala Gly Cys Cys Asn Asp Glu Ala		
80	85	90
CTG GAG TGC GTG CCC ACG TCG GAG AGC AAC GTC ACT ATG CAG ATC	319	
Leu Glu Cys Val Pro Thr Ser Glu Ser Asn Val Thr Met Gln Ile		
95	100	105
ATG CGG ATC AAA CCT CAC CAA AGC CAG CAC ATA CGA GAG ATG AGC	364	
Met Arg Ile Lys Pro His Gln Ser Gln His Ile Gly Glu Met Ser		
110	115	120
TTC CTG CAG CAT AGC AGA TGT GAA TGC AGA CCA AAG AAA GAT AGA	409	
Phe Leu Gln His Ser Arg Cys Glu Cys Arg Pro Lys Lys Asp Arg		
125	130	135
ACA AAG CCA GAA AAA AAA TCA GTT CGA GGA AAG GGA AAG GGT CAA	454	
Thr Lys Pro Glu Lys Lys Ser Val Arg Gly Lys Gly Lys Gly Gln		
140	145	150
AAA CGA AAG OGC AAG AAA TCC CGG TTT AAA TCC TGG AGC GTT CAC	499	
Lys Arg Lys Arg Lys Lys Ser Arg Phe Lys Ser Trp Ser Val His		
155	160	165

FIG. 6A

TGT GAG CCT TGT TCA GAG CCG AGA AAG CAT TTG TTT GTC CAA GAT 544
Cys Glu Pro Cys Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp
170 175 180

CCG CAG ACG TGT AAA TGT TCC TGC AAA AAC ACA GAC TGG CGT TGC 589
Pro Gln Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys
185 190 195

AAG GCG AGG CAG CTT GAG TTA AAC GAA CGT ACT TGC AGA TGT GAC 634
Lys Ala Arg Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp
200 205 210

AAG CCA AGG CCG TGA
Lys Pro Arg Arg 649

FIG.6B

ATG CTG GCC ATG AAG CIG TTC ACT TGC TTC TTG CAG GTC CTA GCT Met Leu Ala Met Lys Leu Phe Thr Cys Phe Leu Gln Val Leu Ala 5 10 15	45
GGG TTG GCT GTG CAC TCC CAG GGG GGC CTG TCT GCT GGG AAC AAC Gly Leu Ala Val His Ser Gln Gly Ala Leu Ser Ala Gly Asn Asn 20 25 30	90
TCA ACA GAA ATG GAA GTG GTG CCT TTC AAT GAA GTG TGG GGC CGC Ser Thr Glu Met Glu Val Val Pro Phe Asn Glu Val Trp Gly Arg 35 40 45	135
AGC TAC TGC CGG CCA ATG GAG AAG CTG GTG TAC ATT GCA GAT GAA Ser Tyr Cys Arg Pro Met Glu Lys Leu Val Tyr Ile Ala Asp Glu 50 55 60	180
CAC CCT AAT GAA GTG TCT CAT ATA TTC AGT CGG TCA TGT GTC CTT His Pro Asn Glu Val Ser His Ile Phe Ser Pro Ser Cys Val Leu 65 70 75	225
CTG AGT CGC TGT AGT GGC TGC TGT GGT GAC GAG GGT CTG CAC TGT Leu Ser Arg Cys Ser Gly Cys Cys Gly Asp Glu Gly Leu His Cys 80 85 90	270
GTG GCG CTA AAG ACA GCC AAC ATC ACT ATG CAG ATC TTA AAG ATT Val Ala Leu Lys Thr Ala Asn Ile Thr Met Gln Ile Leu Lys Ile 95 100 105	315
CCC CCC AAT CGG GAT CCA CAT TCC TAC GTG GAG ATG ACA TTC TCT Pro Pro Asn Arg Asp Pro His Ser Tyr Val Glu Met Thr Phe Ser 110 115 120	360
CAG GAT GTA CTC TGC GAA TGC AGG CCT ATT CTG GAG ACG ACA AAG Gln Asp Val Leu Cys Glu Cys Arg Pro Ile Leu Glu Thr Thr Lys 125 130 135	405
GCA GAA AGG TAA Ala Glu Arg	417

FIG.7

ATG CTG GCC ATG AAG CTG TTC ACT TGC TTC TTG CAG GTC CTA GCT Met Leu Ala Met Lys Leu Phe Thr Cys Phe Leu Gln Val Leu Ala 5 10 15	45
GGG TTG GCT GTG CAC TCC CAG GGG GGC CTG TCT GCT GGG AAC AAC Gly Leu Ala Val His Ser Gln Gly Ala Leu Ser Ala Gly Asn Asn 20 25 30	90
TCA ACA GAA ATG GAA GTG GTG CCT TTC AAT GAA GTG TGG GGC CGC Ser Thr Glu Met Val Val Pro Phe Asn Glu Val Trp Gly Arg 35 40 45	135
AGC TAC TGC CGG CCA ATG GAG AAG CTG GTG TAC ATT GCA GAT GAA Ser Tyr Cys Arg Pro Met Glu Lys Leu Val Tyr Ile Ala Asp Glu 50 55 60	180
CAC CCT AAT GAA GTG TCT CAT ATA TTC AGT CGG TCA TGT GTC CTT His Pro Asn Glu Val Ser His Ile Phe Ser Pro Ser Cys Val Leu 65 70 75	225
CTG AGT CGC TGT AGT GGC TGC TGT GGT GAC GAG GGT CTG CAC TGT Leu Ser Arg Cys Ser Gly Cys Cys Gly Asp Glu Gly Leu His Cys 80 85 90	270
GIG GCG CTA AAG ACA GCC AAC ATC ACT ATG CAG ATC TTA AAG ATT Val Ala Leu Lys Thr Ala Asn Ile Thr Met Gln Ile Leu Lys Ile 95 100 105	315
CCC CCC AAT CGG GAT CCA CAT TCC TAC GTG GAG ATG ACA TTC TCT Pro Pro Asn Arg Asp Pro His Ser Tyr Val Glu Met Thr Phe Ser 110 115 120	360
CAG GAT GTA CTC TGC GAA TGC AGG CCT ATT CTG GAG ACG ACA AAG Gln Asp Val Leu Cys Glu Cys Arg Pro Ile Leu Glu Thr Thr Lys 125 130 135	405
GCA GAA AGG AGG AAA ACC AAG GGG AAG AGG AAG CAA AGC AAA ACC Ala Glu Arg Arg Lys Thr Lys Gly Lys Arg Lys Gln Ser Lys Thr 140 145 150	450
CCA CAG ACT GAG GAA CCC CAC CTG TGA Pro Gln Thr Glu Glu Pro His Leu 155	477

FIG. 8

ATG CCG GTC ATG AGG CTG TTC CCT TGC TTC CTG CAG CTC CTG GCC Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala 5 10 15	45
GGG CTG GCG CTG CCT GCT GTG OOC OOC CAG CAG TGG GOC TTG TCT Gly Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser 20 25 30	90
GCT GGG AAC GGC TCG TCA GAG GTG GAA GTG GTA CCC TTC CAG GAA Ala Gly Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu 35 40 45	135
GTG TGG GGC CGC AGC TAC TGC CGG CGG CTG GAG AGG CTG GTG GAC Val Trp Gly Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp 50 55 60	180
GTC GTG TCC GAG TAC CCC AGC GAG GTG GAG CAC ATG TTC AGC CCA Val Val Ser Glu Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro 65 70 75	225
TCC TGT GTC TCC CTG CTG CGC TGC ACC GGC TGC TGC GGC GAT GAG Ser Cys Val Ser Leu Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu 80 85 90	270
AAT CTG CAC TGT GTG CCG GTG GAG ACG GCC AAT GTC ACC ATG CAG Asn Leu His Cys Val Pro Val Glu Thr Ala Asn Val Thr Met Gln 95 100 105	315
CTC CTA AAG ATC CGT TCT GGG GAC OGG CCC TOC TAC GTG GAG CTG Leu Leu Lys Ile Arg Ser Gly Asp Arg Pro Ser Tyr Val Glu Leu 110 115 120	360
ACG TTC TCT CAG CAC GTT CGC TGC GAA TGC CGG CCT CTG OGG GAG Thr Phe Ser Gln His Val Arg Cys Glu Cys Arg Pro Leu Arg Glu 125 130 135	405
AAG ATG AAG CGG GAA AGG AGG AGA CCC AAG GGC AGG GGG AAG AGG Lys Met Lys Pro Glu Arg Arg Arg Pro Lys Gly Arg Gly Lys Arg 140 145 150	450
AGG AGA GAG AAG TAG Arg Arg Glu Lys	465

FIG. 9



European Patent
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EUROPEAN SEARCH REPORT

Application Number

EP 92 30 2750

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl.)		
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim			
A	EP-A-0 399 816 (MERCK)	1-17	C07K15/06		
Y	* the whole document *	18-20	C12N15/19		
A	WO-A-9 102 058 (CALIFORNIA BIOTECHNOLOGY)	1-20	C07K3/28		
	* the whole document *	---	C12N5/10		
A	WO-A-9 013 649 (GENENTECH)	1-20	A61K37/36		
	* the whole document *	---	//C07H21/00		
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The present search report has been drawn up for all claims					
Place of search	Date of completion of the search	Examiner			
THE HAGUE	08 JULY 1992	MOLINA GALAN E.			
CATEGORY OF CITED DOCUMENTS					
X : particularly relevant if taken alone	T : theory or principle underlying the invention				
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